ALTERNARIA DISEASES OF CABBAGE AND RELATED PLANTS

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A Thesis Submitted for the Degree of

MASTER OF SCIENCE

UNIVERSITY OF WISCONSIN

1917

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I. INTRODUCTION

For a long time the term "leaf spot" has been applied to diseases, on cabbage and related plants, characterized by roughly circular infected areas with pretty sharply defined margins. Usually the term has been applied to diseased conditions supposedly due to Alternaria brassicae. However, other species of Alternaria, and also Macrosporium, Rhopalidium, and Polydesmus have been found on similar spots on the leaves of cruciferous plants; but with few exceptions these fungi have not received critical study, nor has the relation of the fungus as a possible cause of the spots on the leaves been satisfactorily defined in any of these cases. It is doubtful whether the different fungi found on leaf spot can be distinguished by the unaided eye merely from the appearance of the infected areas.

II. PURPOSE

Because of the fact that the leaf spot fungi on cultivated crucifers are not well understood from the morphological, the physiological, or the pathogenic standpoint, the writer has undertaken his research with the following objects in view:

1. To determine whether leaf spot on cultivated crucifers in Wisconsin and neighboring states is due directly to pathogenic fungi; and if so, to which ones.

2. To determine whether the spots due to the attacks

on the leaves of cultivated crucifers by different Alternarias can be associated with definite species by the unaided eye.

3. To determine, as far as possible, the geographical range of leaf spot on cultivated crucifers.

4. To describe the morphological characteristics of the different fungus species associated with these spots, viz. Alternaria brassicae, Macrosporium herculeum, and an undetermined Alternaria referred to in this thesis as Alternaria sp.

5. To determine whether any or all of these fungi are the direct cause of leaf spot.

6. To describe the physiological characteristics of these fungi in culture.

7. To determine factors favorable to infection and progress of the disease.

8. To give methods of inoculation of healthy host plants, and methods used in isolation of the organism.

9. To determine as far as possible the host range of the above fungi.

10. To ascertain the existing relations of these fungi to their hosts.

11. To determine whether any of these fungi are the direct cause of disease on cabbage in storage, and to secure evidence regarding their economic importance.

12. To secure facts relative to the longevity and overwintering of these fungi.

13. To give, as far as possible, control measures for the fungi above mentioned.

III. REVIEW OF LITERATURE

The literature relating to leaf spot diseases of cultivated crucifers is small in amount, scattered, rather confusing, and of but little importance. Most of the literature on leaf spot holds Alternaria brassicae responsible for the disease, but a clear morphological description of the fungus is altogether lacking. The confusion is increased by applying the name Alternaria brassicae to a large number of fungi having similar spore forms, and then attempting to distinguish between them by varietal names. This is evidently due to a lack of careful morphological and physiological study, otherwise more of the Alternarias would be divided into species.

Excerpts from the literature on the subject of leaf spot are given in the following pages, followed by a comparative digest.

Höhnel, Franz v. (4)

"The original sample at the herbarium of Montagne in Paris consists of a faded leaf of Brassicae, upon which an

abundance of blackish-brown spots are found. No sort of a fruiting body of a fungus in the same or on other parts of the leaf are to be found.

Montagne at first described this fungus as Puccinia? Brassicae. He says that the 200 to 250μ long, long-beaked spores invariably lie under the epidermis and that other spores too, more cylindrical, without beaks may be found. That the spores did not lie beneath the epidermis is evident from the fact that on the original sample no trace of spores is to be found. Evidently they lay upon the epidermis and have been since the year 1828. During the 82 years of preservation of the original sample, which was not poisoned nor placed into a capsule, the spores were evidently eaten away by the booklice, which always takes place in case of Hyphomycetes. Had the spores been covered by the epidermis they would unquestionably be present there today. Consequently the fungus is a brown Hyphomycete above the surface. If one now observes Montagne's drawings of these spores it will be seen that plainly the fungus is an Alternaria or a Macrosporium.

"If one compares leaves of Brassicae which are affected with Alternaria Brassicae with an original leaf affected with Rhopalidium, one observes that the spots in both cases are identical. Structure, size, form, arrangement, and number of spots absolutely admit of no difference. Consequently every doubt is excluded that Rhopalidium Brassicae = Alternaria Bras-

sicae. In this species the length, form, and septation of the spores are very variable. Here occur beaked, unbeaked, spindle-form, cylinder-form, short and long, also cross septate and muriform spores. According to Montagne's account of Rhopalidium Alternaria Brassicae Sacc. var. macrospora Sacc. (Syll. Fung. IV, p. 546) answer the description best, which spores in size are 120-140 x 20-25μ, and which present up to 11 cross septa and often one to two longitudinal septa. However, the spores attain greater size, since Macrosporium herculeum Ell. and Mart. has spores up to 225μ in length and there is no doubt that this form is identical to the one before mentioned.

"After what has been said there can be no reasonable doubt that Rhopalidium Brassicae Mont. et Fr. = Alternaria Brassicae var. macrospora Sacc."

Ferraris, J. (2)

"Alternaria brassicae Sacc. is a species very common on the leaves of herbaceous plants, and occurs on a diverse variety of them. Of these it is on the variety exitiosa (Polydesmus exitiosus Kühn.) with the conidial form Leptosphaeria napi (Fuck.) Sacc., of which we have spoken on p. 422, considering it an alteration in the form fructification differing from that on crucifers (Raphanus, Sinapis, Brassica Rapa, Brassica Napus, etc.).

"The type and variety Macrospora produce there a notable disease of the leaves of cabbage and cauliflower of Cochlearia Armoracia and other crucifers, wild and cultivated. This disease manifests itself as round spots on the leaves, at first

small, then increasing up to a centimeter in diameter, olivaceous, and with concentric zones, very distinct, of a brown color. The mycelium develops in the parenchyma of the leaves, and, breaking out at the stomata with fasicles of conidiophores, develop large, olivaceous conidia, obclavate or fusoid, straight or slightly curved, with transverse and longitudinal septa, and terminated with an appendage which is much more hyaline up to the point where another smaller conidium may be inserted. A third conidium may be attached to the second, and so on. The larger conidia may measure 120-140 x 14-25μ. The leaves attacked by this disease are generally the oldest or lowest on the stem, and they dry out rapidly."

The illustration shows two longitudinal septa in the larger or lower spore, and a small spore about one-fifth the length of the larger spore at the end of the beak. The beak of the larger spore is about the length of the spore proper. Neither the description nor the illustration indicates that the fungus Ferraris had in mind is the same as the fungus common on our cultivated crucifers known as A. brassicae.

Referring to other fungi Ferraris says on p. 889: Macrosporium commune Rabh. and Macrosporium sarcinula Berk. are two very common saprophytes which are also able in certain cases to exercise a parasitic action on organs of herbaceous plants (asparagus, onions, garlic) causing on the organs attacked a characteristic ring effect. They are conidial forms of Pleospora herbarum.

Hollrung, M. (6)

"According to observations made by Ferraris there appears a disease on Phaseolus vulgaris var. nanus, irregularly distributed, yellow in the beginning, later brownish-red, and confluent spots. Upon further progress it causes dying and falling of the leaves. On the latter there was found Alternaria brassicae forma phaseoli P. Brun. By microscopic observations Ferraris followed the mycelium in microscopic cross sections and the exit of the conidiophores through the stomata. The affected parts became hypertrophied (normal leaf 130μ, affected 240-250μ thick). Above all the palisade layer is much enlarged. The observer concludes that Alternaria is to be regarded as a parasite. Favorable to the progress of the disease after its appearance several showers of rain fell, with a rise in temperature, in June to the middle of July. In the places when the beans were not exposed to the full sunlight the fungus appeared most abundant. Bordeaux mixture and ammonical copper carbonate were recommended as a remedy. A description of the fungus was given".

Hollrung, M. (6)

"The disease due to Alternaria brassicae var. nigrescens manifests itself in the appearance of round spots on the leaves."

"The appearance of the disease due to A. brassicae var. nigrescens may be retarded by the use of Bordeaux mixture, and likewise by the use of a solution of liver of sulphur. These

at the same time serve as beneficial food material for the melons."

Sheldon, J. L. (11)

Leaf-mold or Blight (Alternaria brassicae var. nigrescens).

"The fungus which causes this disease produces yellowish-brown spots on the leaves of muskmelon and cucumber similar to those produced by the downy mildew in color, but usually thicker, and with more or less distinct wavy markings. There was scarcely a garden or field containing muskmelons which I visited where I did not find this disease. A field of muskmelons, where the fungus was very destructive, had a few hills of cucumbers. In other fields where there were few hills of muskmelons among cucumbers the disease was found on the muskmelons. The injury done to the cucumbers was in all cases slight..... Upon inquiry from a number of growers, I learned that while the yield is considerably lessened by the disease, the quality of the muskmelon is so poor, especially of the last to ripen, and they are practically worthless. This disease can be controlled to a considerable extent by spraying. As a means of prevention, it is not advisable to plant muskmelons on the same ground the second year. Whether this disease will become as injurious to the cucumber, in the vicinity of Point Pleasant where cucumbers are grown for pickles, remains to be determined."

Sorauer, Paul (12)

"Regarding an intensive appearance of A. brassicae on

cabbage plants, G. Arcangeli says (in Bullett d. Soc. botan. Italiana; Firenze, 1898; S. 180):

"As early as in the first half of April spores of the fungus manifest themselves in the cauliflower gardens of Eben von Cascina near Pisa. The parasite, however, did not confine itself to the leaves alone, but also showed black spots on the flower buds, on account of which the plants were refused on the market."

Sorauer, Paul (13)

"During a number of years the melon crops have occasionally been inferior, and at times total failures. This is due to three causes, viz., attacks by Bacillus tracheiphilus, sometimes in combination with a Fusarium fungus; Alternaria brassicae, var. nigrescens; and tip burn. ---- Spraying with sulphur burns the leaves, weak Bordeaux mixture and lime sulphur used as preventive measures against infection due to Alternaria brassicae may be regarded as favorable."

In the same volume, 1901, p. 232, is found a short article on Plant Diseases in Italy. The fungi spoken of as being especially detrimental to cultivation are: Cycloconium oleaginum Cast. (on olive tree), Fusicladium pyrinum Fuck.(on pear tree), Alternaria brassicae Sacc. f. nigrescens Pegl. (melons).

The above is probably not the same as Alternaria brassicae usually found on crucifers.

Voglino, P. (17)

"Circular or irregular spots appeared on the leaves

of cauliflowers, while the inflorescence became yellow in a number of places and then dried up upon turning black. The same infection had appeared in the vicinity of Verona in 1901 and was attributed to Polydesmus exitiosus Kühn. which the author upon the evidence of his own culture identified as Alternaria brassicae (Berk.) Sacc. Solla."

Voglino, P. (18)

"On the plants of cauliflower at Verona the author was able to identify the presence of Polydesmus exitiosus Kühn. The size of the conidia and the number of crosswalls were not always comparable, as Kühn, too, represented them in his drawings.

"On the leaves of cabbage at about the same time Alternaria brassicae (Berk.) Sacc. was observed. His own cultures of both fungi and a study of the mycelium as well as finally artificial inoculations confirmed that Polydesmus exitiosus and Alternaria brassicae are identical. Solla."

Salmon, E. S. (11)

Leaf Spot of Cabbage and Broccoli - Mycosphaerella brassicicola (Duby) Lindau.

"The fungus frequently found during the winter months on leaves of species of Brassica (in plate XVIII), is considered to be the 'perfect stage' of a conidial form Phyllosticta brassicae which causes a 'leaf spot' on Cabbage, Broccoli, and allied plants. Dr. M. C. Cooke in his 'Fungoid Pests of Cultivated Plants' says of this fungus: 'The mature stage of this

pest, in the form of Sphaerella, is not reached until the leaves have lain some time on the ground.'

"Observations made at this college during February and March, 1914, show that this statement requires modification, for in some cases the Sphaerella (or Mycosphaerella) stage with mature ascospores has been found on living green leaves of cabbage and broccoli. On one occasion, while examination of the fruit bodies was being made under the microscope, the asci were seen to elongate and eject the ascospores. The spores as shown in Fig. 2 are two-celled, spindle-shaped (fusiform), with rounded ends, and with a slight constriction at the septum; the septum is medial, but as a rule one cell is slightly broader than the other.

"They are usually thrown out in rapid succession, though sometimes a few seconds elapse between the ejection of one spore and that of the next. While the process was under observation, one spore was caught at the middle where the constriction occurs, by the partial closing of the pore of the ascus, so that one of the two cells of the spore was outside and the other inside of the ascus, it remained in that position for a short time but was eventually ejected.

"Green leaves of Broccoli, with the mature stage of the fungus have been sent in from Clapham, near Worthing, and from Hancross, Sussex. Our correspondent from Hancross writes: 'I enclose some specimens of Broccoli leaves (variety, Late Queen), The "spot" appears in autumn and increases in extent

during winter, attacking all but the younger leaves of most of the Brassicas we grow; I have not noticed it on turnips. The disease is very prevalent all over the neighborhood, especially in the allotments."

"Living leaves on Broccoli and Cabbage obtained from the College gardens were also found to have the Mycosphaerella stage (with mature spores) on the diseased spots."

Stevens, F. L. and Hall, J. G. (16)

Black mold (Alternaria brassicae (Berk.) Sacc.)

"The affected spots are nearly black, marked concentrically, are circular, and are not definitely bordered, i. e. they shade off gradually into the surrounding healthy tissue. They enlarge sometimes to 2-3 cm. in diameter. The tissue dries, becomes brittle, and often falls away, leaving ragged holes.

"The general appearance of the spot as seen from above is pale green; as seen from the lower surface, shown in Fig. 108, it is densely black, strongly contrasting with the white spots of the downy mildew which may occur in association with this disease. The damage may be very great, in many instances resulting in the death of the plants or complete loss of their usefulness."

Stevens, F. L. (14)

A. brassicae (Berk.) Sacc.

"Conidiophores short, continuous, short-branched, apically equal, conidia elongate, fusoid, clavate, 60-80 x 14-18μ, 6-8 muriform septate, olivaceous. On crucifers."

Stevens, F. L. (15)

"M. herculeum E. & M. Amphigenous, on rounded, grey spots; conidiophores erect, cespitose, flexuous, brown, few septate, 70-80 x 5μ; conidia brown, multiseptate, clavate, 200-225 x 21-26μ."

"It causes leaf spot on turnips, horse radish and other crucifers."

Harter, L. L. (3)

Leaf-Blight (Black mold)

"Description.- Leaf blight is due to the fungus Alternaria brassicae (Berk.) Sacc., which may attack the plant at any stage of its growth. The vegetative part of the fungus lives in the leaf tissue of the host and under field conditions forms roundish black spots marked with concentric brown zones. These spots vary from one-fourth to one-half inch or more in diameter. The fungus may also live as a saprophyte and causes considerable damage to cabbage in storage houses.

"Control.- To prevent loss from this fungus in the storage house the following suggestions should be observed:

(1) disinfect the storage house by spraying the walls, benches, and bins with Bordeaux mixture; (2) exercise care in handling, so as to minimize injury to the heads; (3) maintain a temperature 1 or 2 degrees above freezing, and (4) keep the humidity as low as possible by proper ventilation of the house with outside air.

"Distribution and loss.- Leaf blight causes considerable damage to cabbage and collards in this country and in Europe. The greatest loss to cabbage occurs in the storage house. The organism causing the disease is present in the houses under ordinary conditions, or it may be carried to these with the cabbage when it goes into storage. It gains access to the tissues through wounds made by handling and cutting or by following up the tissue killed by other organisms. In the presence of plenty of moisture and a suitable temperature it develops rapidly, forming an unsightly black mold over the heads."

Rolfs, P. H. (7)

"Leaf spot (Alternaria brassicae).- This is one of the minor diseases of cabbage, although at times it may cause much damage. It appeared rather severely in one locality on low damp soil. The fungus causing the disease spreads out from a definite point upon the leaf, and produces large, dark, irregular spots, which later are covered with a felt-like coat of spores. The fungus may destroy the growth of the plant, or at a later time may destroy the leaves of the head so as to render it worthless for market."

Bessey, C. E. (1)

"Macrosporium herculeum Ellis and Martin. Amphigenous, on dark gray, round spots; hyphae erect, brown, caespitose, flexuous, sparingly septate, 70-80 x 5μ; conidia brown, clavate, multiseptate with a few imperfect longitudinal septa, 200-225 x 21-26μ. On leaves of Nasturtium Armoracia. Newfield, N. J."

Saccardo, P. (8)

"Macrosporium herculeum Ell. and Mart. Amphigenous, spots sorted, gray, round. Conidiophores erect, tufted, flexuous, brown, with few septa, 70-80μ. Conidia brown, clubshaped, many septate, few longitudinal septa, 200-225 x 21-26μ. On leaves of horse radish. Newfield, N. J."

Saccardo, P. A. (9)

Alternaria brassicae (Berk.) Sacc. The hyphae are short and continuous, with short, equal branches near the tip, tufted. Conidia cantenulate, soon deciduous, elongate, fusoid, clavate 60-80 x 14-18μ, at first continuous at length muriform, 6-8 septate, olivaceous.

From the above review of literature the reader must come to the conclusion that there is a necessity for a more thorough study of fungi causing leaf spot on the garden crucifers, not only because of the diseases of these plants but because of another factor of equal importance, - the relation which the Alternarias may bear to fungus diseases on other plants. In order to make a study of such relations as may exist between diseases of plants of different genera it is of the utmost importance that the species of fungus under consideration

be definitely determined. No such determinations can be made from the above literature. That the different species of Alternaria are not well understood is well illustrated in Höhnel's article (4) where one would get the impression that Alternaria brassicae Sacc., Rophalidium brassicae Fr. et Montagne, and Macrosporium herculeum E. and M. are one and the same fungus. These conclusions are drawn from mere spots on leaves some eighty years old on which no spores were found, from the preliminary description of M. herculeum, and from an incomplete description of A. brassicae.

Another illustration of the uncertainty of the identity of these species of fungi is found in Sulla's articles in Zeitschrift für Pflanzenkrankheiten (17, 18), where what was supposed to be Polydesmus exitiosus was found to be Alternaria brassicae. Unfortunately the author gives no detailed description of the fungus.

Another unfortunate feature of the literature is that in most cases the fungus is reported as being found on certain plants, or as causing leaf spot on certain plants without giving definite proof of the pathogenicity of the fungus. Many fungi may be found on plants without being the primary cause of the disease. As an illustration the article by Hollrung (6) may be considered. Hollrung says that Ferraris found "Alternaria brassicae forma phaseoli" on Phaseolus vulgaris var. nanus, and that Ferraris concludes that the fungus must be regarded as a parasite. However, Hollrung gives no experimental evidence upon

which such conclusion is based, nor has the writer been able to find any literature left by Ferraris which shows that if such conclusions were drawn they were based on experimental evidence.

The literature regarding Macrosporium herculeum is very meagre and indefinite. Stevens (16) does not mention the mycelium, and in defining the conidia says they are multiseptate but fails to state that they are muriform-septate. Saccardo's (9) description is similar. Bessey (1) mentions imperfect longitudinal septa in the spores, but says the hyphae are sparingly septate.

The description of the spots resulting from the attack of the various fungi on the whole are misleading. The spots caused by A. brassicae (Berk.) Sacc. (4, 8, 17) are usually given as being from one-fourth to one-half inch or more in diameter, but the extremes are not mentioned. The confusion caused by the omission of the extremes is illustrated by a quotation taken from a letter by I. C. Jagger, Rochester, N. Y., who made a valuable contribution to the writer's collection of specimens. The contribution was made in three packages carefully labeled. Mr. Jagger says in part:

"Specimens No. 3 are the fine head spots, the outer leaves of the hard head which we observed at several places last summer. Specimens No. 2 are very dark leaf spots which may be the early stages of Alternaria spots although I have considerable doubt."

Without much difficulty, the writer, by the fragment

method, isolated A. brassicae (Berk.) Sacc. from a number of spots of each of the samples No. 3 and No. 2. The fact that an experienced plant pathologist like Mr. Jagger was in doubt as to the cause of the very dark spors on the leaves in package No. 2, and that apparently he had no idea that the small specks on the leaves in package No. 3 were due to A. brassicae suggests that the common descriptions of the spots caused by this fungus as they occur in literature are misleading and do not serve the purpose.

There is but little in the literature relating to the economic importance of the leaf spot fungi. The following authors give rather vague data as to the seriousness caused by various fungi attacking cultivated crucifers. Sorauer (12), Salmon (10), Stevens and Hall (16), Rolfs (7), and Harter (3). Harter (3) is the only author who suggests practical control measures to prevent damage due to A. brassicae in storage.

In a communication received from M. H. Vaughan, South Haven, Mich., it appears that he is encountering considerable difficulty in growing cauliflower on account of leaf spot, attack of which is most serious between the time the young plants are transplanted and when they are about half-grown. The disease occurs almost yearly. C. M. Frey, acting as county representative, states that E. Bessey, East Lansing, Mich., identified the causal organism as A. brassicae. No serious trouble was experienced in the growing of cabbage.

SOURCE OF MATERIAL

The writer is indebted to a considerable number of men at various experiment stations for material, chiefly cabbage leaves, furnished for the study of leaf spot. The following are the chief sources: Atlanta, Ga.; Ames, Iowa; Wooster, Ohio; Farmingdale, Long Island; Rochester, N. Y.; Burlington, Vt.; Norfolk, Va.; St. Paul, Minn.; Morgantown, W. Va. From Wisconsin, samples were secured from the following places: Madison, Racine, Wausau, and Portage. Cabbage leaves were also secured from D. M. Ferry Co., Detroit, Michigan.

Isolations were made by the spore dilution method and by the fragment method. In this way two species of Alternaria were secured and given critical study.

MORPHOLOGY

Alternaria brassicae

Most of the leaf spot of cabbage prevalent throughout a large part of the United States during the fall of 1915 was evidently due to the fungus corresponding closely to Alternaria brassicae as described by Saccardo (9) and Stevens (15). This refers to the fungus to which no variety name has been attached. From nine widely different sections of the country the same fungus was isolated.

Mycelium. The mycelium as seen in artificial cultures on potato agar is at first hyaline, but after several days becomes slightly dark, and in time becomes dark brown. The mycelium is 4 to 6μ in diameter, much branched, grows to a considerable length, and septa occur at short intervals.

Conidiophores. The conidiophores grow mostly, although not exclusively, at right angles to the mycelium. They usually, but not necessarily, arise near the tips of the hyphae, or they may be terminal. Sometimes they appear nearly opposite one another. They are usually from 3 to 5μ in length and are then non-septate. However, they may attain a length of from 10 to 40μ, and may be once or twice septate. The conidiophores are equal in diameter to the mycelium. They are hyaline at first, but become dark brown with age.

Conidia. The conidia vary greatly in forms and size. In length they may measure from 12 to 90μ, in diameter from 6 to 28μ. An average of the largest spores found on cabbage

leaves secured from ten different sources in the fall of 1915 was as follows:

B. B. Higgins, Experiment, Georgia	64.4 x 16.8μ
C. L. Fitch, Ames, Iowa	63.4 x 17.2
J. G. Humbert, Wooster, Ohio	55.2 x 14.4
A. A. Johnson, Farmingdale, Long Island	62.8 x 19.0
I. C. Jagger, Rochester, New York	64.0 x 19.6
B. L. Lutman, Burlington, Vermont	65.4 x 15.0
J. A. McClintock, Norfolk, Virginia	65.2 x 24.0
N. J. Giddings, Morgantown, West Virginia	70.8 x 15.4
R. E. Vaughan, Racine, Wisconsin	67.6 x 14.4
University of Wisconsin Experimental Plot	69.6 x 17.0

From the above it is apparent that it is useless to attempt to distinguish A. brassicae by spore measurements. It is equally useless to attempt to find an average with a view to guide the reader to distinguish the fungus from other Alternarias by spore measurements. Suffice it to say that the largest spores average between 60-80 x 14-18μ. When young they are of a uniform light brown color, but turn somewhat darker with age. The number of septa may vary from one to twelve. Muriform septation is common in old, well-developed conidia. The cross septa are usually nearly at right angles and the longitudinal septa usually nearly parallel to the long axis of the spores. This is by no means universal, but these conditions prevail much more uniformly in A. brassicae than in many similar saprophytic Alternaria. The cells formed as a result of longitudinal septation

may in turn have cross septa. In form the conidia are elongate, fusoid, clavate; those having longitudinal septa are usually large in diameter and diverse in form. They may be continuous at first, or septate from the beginning as described below. The conidia can scarcely be said to have a beak, although in exceptional cases the terminal cell is elongated and even septate so that a beak is present. They occur in chains of from five to seven, but more may be present. The chains may be branched. From one of the cells of one of the older spores, i. e. one of those near the conidiophore, a branch chain may be produced. Exceptionally two chains may arise from two very short terminal conidiophores as illustrated in Fig. 1 where a bud suggests the beginning of one of two chains. This condition may be mistaken for a branch chain.

Spore formation. Spore formation was studied as follows. One-half of the impression of a culture slide was filled with potato agar under sterile conditions and allowed to cool. A vertical cut was then made across the impression so as to secure a vertical surface, and the superfluous agar was removed. A small number of spores of A. brassicae were then placed at the upper margin of the cut agar surface and a sterile coverglass was placed over the top. The slide was then put into a petri dish which was placed at room temperature.

Spore-formation commenced in forty-eight hours. Just how the first cell is produced from the conidiophore has not been observed, but there is reason to believe that it occurs by

the process of budding. The initial cell has been observed under high power when it was not more than one-third normal size, and has repeatedly been seen to enlarge. At this stage of development the cell is hyaline and ovoid. This form is retained until the cell is full grown at which time it measures approximately 20μ in length and 12μ in diameter, but the size may vary considerably. From the terminal end of the initial cell a hyaline, globular bud not more than 2μ in diameter is thrown out. This bud enlarges rapidly, and in about an hour attains approximately the size and shape of the parent cell. The second cell throws out a bud at its terminal end,- hyaline, globular, and not more than 2μ in diameter. This bud, too, rapidly enlarges until it attains a size slightly smaller than the second cell. Meanwhile, the walls between the first and second cells are compressed so that only a single cross-wall or septum is visible. The same thing occurs between the second and third cells. The first cell now becomes slightly yellow. The second cell in turn becomes slightly yellow while the first takes on a brownish tinge and may form a cross septum. The third cell then becomes slightly yellow while the second becomes brownish. The third cell finally becomes brownish with the exception of a small hyaline spot at the terminal end, which has by this time become bluntly pointed. The spot, nearly hyaline, at the tip usually or at least very often, persists in the spore. The total time required for the formation of a spore, not including subsequent septation, is approximately four hours.

The second spore in a chain is formed as follows: A bud precisely the same as those above mentioned is thrown out from the terminal end of the third cell of the first spore. The bud enlarges, becomes elliptical in form, turns slightly yellow, first at the proximal end and then gradually toward the terminal end, forms a cross septum near the center, becomes bluntly pointed at the terminal end, and then turns brown. More septa may then be formed. It appears that the second spore does not as a rule attain as great a size as the first, and the same thing appears to be true of subsequent spores in a chain.

The first spore on a conidiophore is not always formed by the addition of one cell to another by the process of budding. This seems to occur only when vigorous growth is taking place. Otherwise the first spore is produced as described under the formation of the second spore. The various steps in spore formation are illustrated in Fig. 2. The conidiophore at the end next the spore is rounded. This is also true of the proximal end of the spore. The point of attachment is small. The same conditions prevail between two spores in a chain. The spores are not very easily broken apart when young, and they are rather firm when young as well as when old.

The experience of the writer suggests that the young bud as it emerges from the terminal cell does not resume its growth under the microscope when the light is passed through for a long time. When the microscope, with the slide in position, has been standing in the dark, as in the microscope case,

the buds emerge from the cell wall readily. The microscope may then be removed from the case for a few seconds and the process may be watched. In several cases the bud was permanently stopped in development when exposed to the light for two or three minutes. The bud emerges from the cell wall in approximately three to six seconds. After the bud has attained considerable size it is not so easily stopped in development. These statements regarding the effect of light should not be taken too seriously. More work on the subject is required to form definite conclusions.

Macrosporium herculeum

A fungus which corresponds closely in description to Macrosporium herculeum E. and M. as described by Bessey (1), Saccardo (8), and Stevens (15) was isolated from a cabbage leaf from Wooster, Ohio, from a cabbage leaf from the experimental garden of the University of Wisconsin, and from a rutabaga leaf from Wausau, Wisconsin.

Mycelium. On potato agar, as observed by use of a culture slide under the microscope, the mycelium is much branched, abundantly septate, and from 5 to 8μ in diameter. Very fine, dark granules appear in the mycelium from the beginning. Branching of the mycelium takes place in a rather singular manner. The terminal end ceases in growth and a pear-shaped body nearly twice the diameter of the mycelium is formed terminally, the smaller end being next the mycelium. After resting for a periodof

about ten hours, two or three branches arise from the pear-shaped body and continue to grow rapidly, about 60μ per hour. The enlargements are numerous throughout the mycelium.

Conidiophores. How the conidiophores arise from the mycelium has not been observed. A constriction appears in the conidiophore where it joins the mycelium, the point of attachment being about half the diameter of the conidiophore. A septum occurs in the mycelium on either side of the conidiophore as illustrated in Fig. 3. The conidiophores are from 4 to 7μ in diameter, and slightly enlarged at the terminal and proximal ends. They may be straight, slightly bent, or flexuous, and usually from 70 to 160μ in length. They are granular in appearance.

Conidia. The mature conidia as found in nature (Fig. 4) are uniformly very light brown, clavate, usually slightly curved, 9 to 12 septate, with occasional spores having one or two longitudinal septa. The proximal end of the spore tends to be bluntly pointed. Diverse forms of spores are occasionally found. The length of the conidia varies from about 80 to 230μ, though greater extremes may be found. The beak is approximately equal in length to the spore proper. The average of the largest spores is 200 to 225μ. Very short spores, or spores without a beak, are seldom found. The diameter of the spores varies from about 15 to 30μ, the average of the larger ones being 21 to 26μ. They are rather fragile, and many are found broken. On a slide in a drop of water they are so eas-

ily broken by a dissecting needle.that their identity may be destroyed. Some conidia germinate readily in tap water, while others are slow to germinate. No other spore form has been found.

Spore formation. On potato agar on a culture slide the young conidium appears at the end of the conidiophore as an ovoid, granular bud which soon becomes elliptical, enlarges rapidly, and becomes constricted at the center. At the terminal end the cell grows out into a beak. Cross septa are then formed in the conidium, and the outline becomes irregular due to constrictions where the septa meet the outer walls. The various steps in spore formation are illustrated in Fig. 5. A conidium showing a longitudinal septum is shown in Fig. 6. The writer was able to observe detailed spore-formation in only one case. Either the conidia or the conidiophores seem to be heliotropic. As spore formation proceeds the terminal end of the bud turns down toward the light when the culture slide is in position for observation under the microscope.

Indications in culture are that the second spore in a chain is produced at the end of the beak of the first spore by the process of budding,(Fig. 3). Although considerable work was done relative to this matter, the writer has not come to definite conclusions. However, long, elliptical, nearly hyaline, immature spores have often been seen at the ends of the first spore. Chains of two mature spores are common in artificial culture, but more than two spores have never been found.

The beak of the second spore is usually shorter than that of the first spore, Fig. 7. The writer is not aware of the formation of the second spore of a chain during the daytime, but he frequently found them in the morning. Chains of two spores have also been seen on the diseased spot of a cabbage leaf artificially inoculated and placed in a moist chamber. Consequently the fungus is in reality an Alternaria, and the name Alternaria herculeum is probably appropriate.

Alternaria Sp.

While studying the two species of Alternaria above mentioned the writer frequently observed a third Alternaria, the spores of which closely resembled those of A. brassicae in many respects. Though the search is not complete, the writer is inclined to believe that the fungus has not been described so as to distinguish this species from similar Alternarias. The fungus was isolated from leaves from several university plots, from several fields in the vicinity of Portage, Wis., and from material sent from Detroit, Mich. The hosts from which the fungus was isolated are: horse-radish, cabbage (Early Jersey Wakefield, Hollander, and Red), broccoli, collards, kohlrabi, savoy, and Chinese cabbage. The fungus is especially abundant on horse-radish. In a number of cases on acidified potato agar pure cultures of this fungus were secured from spores taken from the diseased spots, and also from fragments from spots on which no conidia could be found,

Mycelium. On potato agar on the culture slide the mycelium makes a profuse growth. The hyphae grow rapidly, about 40μ per hour. At intervals the hypha stops in growth for a short time, forms an enlargement at the end, and from the enlargement a hypha at first rather small in diameter continues to grow in the same direction as previously. The enlargements are frequent throughout the mycelium and a septum is always present at the terminal end. The different hyphae measure from 3 to 7μ in diameter, but the smaller ones grow larger as they become older. The hyphae taper gradually toward the free end. Frequently constrictions occur at the septa. Branches usually at right angles to the main mycelium, or nearly so, are numerous, and are at first small in diameter. The mycelium is granular from the beginning, gradually turns darker, and in a few days becomes gray. In nature the mycelium is rather long, abundantly septate, gray, 4 to 6μ in diameter, and contains enlargements at intervals.

Conidiophores. On the culture slide the conidiophores are usually flexuous, but may be straight. They may be equal, smaller, or greater in diameter than the mycelium from which they arise. They vary from 3 to 6μ in diameter, and from 12 to 70μ in length. At first they are finely granular, but turn to various shades of brown within twenty-four hours. In nature the conidiophores are from 20 to 28μ long, 5 to 6μ in diameter, bent or straight. They are dirty brown in color, and arise in groups from the epidermis of the leaf.

Spore formation. On the culture slide on potato agar the conidia appear to be produced from the conidiophores by the process of budding. The bud, when about one-third the size of a mature conidium, appears finely granular. At this stage it is ovoid in form. It soon becomes elliptical, then bluntly pointed at the terminal end. Meanwhile, one or two cross septa develop. After this the terminal end grows out into a slender beak about equal in length to the spore proper. The color of the spore then changes to various shades of light yellow, and more septa are formed,- cross and longitudinal. Fig. 8. A bud appears to be thrown out at the terminal end of the beak, which develops into the second spore of a chain similar to the first spore. With age the color of the conidia becomes dirty brown, and finally olive brown or nearly black. In the majority of cases the conidia do not possess a beak. The cause of the presence or absence of a beak requires further study. When no beak is present the conidia are often ovate in form, large at the proximal end and gradually taper toward the terminal end. Fig. 9. In nature the conidia are clavate or ovate, with or without beak, muriform septate - the septa frequently being at an angle to the long axis of the spore -, 25 to 110 x 12 to 22μ, the largest ones averaging about 70 to 90 x 15 to 20μ, in chains usually from five to seven. Side chains are produced, but they were usually found to be short. The conidia vary in color from light brown to olive-brown or nearly black when old. The olive-brown is the prevailing color.

RELATION OF FUNGUS TO HOST TISSUE

The writer has not made much study of the relation of the parasite to host. It seems best at this point, however, to summarize what has been learned.

Penetration of germ tube. In an attempt to determine how the mycelium gets access to the leaf tissue, a series of cabbage plants for each A. brassicae and M. herculeum was prepared. The plants were inoculated by placing drops of water spore suspension on definite marked areas on the uninjured leaves. At intervals of 24, 48, 60, 75 and 120 hours six inoculated leaf areas of each series were cut out, three being placed in a vial containing equal parts of glacial acetic acid and alcohol, and three in a vial containing a saturated solution of chloral hydrate. These vials were allowed to stand in the laboratory for ten or fifteen days, and the fragments were then examined under the microscope. The conidia of both fungi showed abundant germination and infections were numerous. In no case for either of the fungi was there a germ tube found which had penetrated through a stoma; nor was there any clear evidence of the penetration of the germ tube through the walls of a living cell or between such cells. The germ tubes were frequently found passing over the stomata, and there were no signs indicating that they tended to seek these openings. It was frequently found, in case of both fungi, that infection had started beneath a spore or group of spores. In such cases the spores nearly al-

ways showed considerable, or almost complete, disintegration. Apparently some substance of a toxic nature associated with the decaying spores had killed some of the cells of the leaf and at the same time these host cell walls had been so changed as to allow the hyphae to penetrate the host tissue.

Mycelium in the leaf tissue. In the leaf tissue the hyphae appear to lie between the cells, but do not seem to penetrate far beyond the diseased area visible to the unaided eye. Whenever there is an indication of infection the identity of the cells is so far destroyed that the cell walls are scarcely distinguishable. Both fungi seem to have this same effect on the host tissue.

Emerging of conidiophores. The writer has not ascertained how the conidiophores emerge from the host tissue in case of either of the fungi, nor has he made a persistent attempt to do so. From observations it does not appear that the conidiophores of either of the above fungi under consideration arise exclusively through the stomata. Apparently they may arise from any place on the leaf surface. At the time the conidiophores appear the leaf tissues are usually so completely destroyed that the stomata cannot be distinguished from the surrounding cells. On a limited number of slides containing cross sections through minute areas attacked by A. brassicae it was found that the cells were totally destroyed where the infection had but little more than started. On the whole the writer must consider his work on this phase of research too incomplete to be satisfactory as a basis for final conclusions.

CULTURAL CHARACTERS

Alternaria brassicae

Plate cultures. Alternaria brassicae grows readily on acidified potato agar. On plate cultures colonies become visible in two days after inoculation. The colony starts as a light-colored mass of mycelium and makes rather rapid growth. On the third day after the plate is poured conidia may be seen at the center of the circular colony which is about one centimeter in diameter. At this stage the colony with its young conidia appears greenish-brown, but later becomes brown. As the colony enlarges conidia are formed farther and farther from the center so that the outer zone of spores is always about four millimeters from the margin of the colony. Meanwhile zonation takes place in the colony. (Fig. 10). When viewed from above the surface of the colony appears densely covered with conidia. Only a few scattered mycelial hyphae at times may be observed on the surface. The agar is seen through the bottom of the plate is not discolored.

Tube cultures. The growth in tube cultures is very similar to that on the plate. In a week the entire slope is covered with a uniform layer of light brown conidia. With age this layer becomes darker and darker in color until in very old cultures it appears nearly black. The individual conidia are then olive-brown. In case of old cultures scattered grayish mycelial threads are usually found superficially over the lower half of

the slope. Potato agar is not discolored, and the mycelium does not appear to penetrate to a great depth. So constant have been the characteristics in hundreds of tube cultures that the writer considers this to be the safest single method to distinguish this fungus from other Alternarias. The spores are readily removed from the slope with a needle, and spore suspensions are made without difficulty.

Macrosporium herculeum

Plate cultures. On plates containing acidified potato agar as a medium the first colonies of Macrosporium herculeum become visible in about five days. At first a few scattered white hyphae appear. This is followed by a dense, tough, pure white mycelial mat which becomes considerably raised above the surrounding medium. The elevated mat grows to be about three or four millimeters in diameter, and from it white mycelial hyphae radiate until they extend to about two centimeters from the center. A broad elevated ring around the center at a distance of about one centimeter is formed. This ring consists of a dense, tough, continuous mat of the same composition as the elevated central portion of the colony. From the ring grow radiations of white mycelial hyphae extending to a distance of about two centimeters from its circumference (Fig. 11), when at a distance of about two centimeters from the center of the colony another ring as just described is formed. This formation of alternate radial rings and broad elevated rings may be

repeated a number of times if the medium is not allowed to dry out. When the second ring is formed the mycelium in the elevated central portion and the first ring become slightly grayish in color and the elevation becomes less. If the agar is allowed to dry out conidia are produced in from ten days to two weeks, more abundantly on the dense rings than between them. However, if the plate is kept in a moist chamber no conidia are developed. The conidia, when present, are close together and more or less entangled in the mycelium. When viewed from above they appear as a very light brown layer which becomes somewhat darker as the colony becomes older. On the whole the colony is interesting as well as beautiful.

Tube cultures. On potato agar slopes, growth is at first very similar to that on the plate. The surface becomes zonated by mycelial ridges. Spore formation begins about two weeks after inoculation. The pure white mycelium spreads, and in about four weeks covers the entire slope. When conidia are first formed they may be removed with a needle for transfer or for spore suspension purposes. However, they soon germinate, and a white, tough, undulated mycelial mat forms over the top. The mycelium turns light gray to dark gray in portions, while in some spots it remains nearly white even in old cultures. The surface often becomes much contorted. Beneath the undulated mycelial mat, in old cultures, is found a tough, leathery layer, about two millimeters thick, which consists of a portion of the culture medium intermixed with a dense mycelium. The leathery

layer is usually bluish purple in color. Beneath this layer the soft potato agar to a depth of from five to ten millimeters is usually bluish purple, but sometimes the potato agar is brownish purple in color. The writer is not certain as to the cause of the difference in color, but it may be due to some variation in the culture medium.

In case of old cultures the only place from which spores can be secured is at the top of the slope where the agar became dried out before the spores have a chance to germinate. Consequently old slopes are not well suited for making a large quantity of spore suspension. In fact, it is rather difficult to get a good spore suspension at any time for the reason that the mycelium is much interwoven with the conidia.

Alternaria sp.

Plate cultures. On potato agar in plate cultures made from a spore suspension the colony becomes visible within forty-eight hours. The colony grows rapidly, attaining a diameter of about an inch in five days from the time the plate is prepared (Fig. 12). Viewed from above the colony is gray in color, and fluffy due to the long mycelium. When from two to four days old the agar, viewed from below, appears dark blue. When five or six days old the blue color at the center gives way to a gray color, but a bluish ring near the margin of the circular colony is present. At this time radiations from the center

to the margin are noticeable. In case of older colonies light gray masses of mycelium, irregular in outline, form over the top. The conidia are produced beneath the fluffy mycelium and are not formed during the first three or four days after the plate is prepared.

Tube cultures. In tube cultures on potato agar the growth of the fungus is very similar to that in plate cultures. a profuse grayish growth covers the entire slope. After the culture is from five days to a week old a dense dark layer beneath the grayish mycelium contains countless conidia. By picking apart a portion of this dark layer in water a spore suspension may be secured. The mycelium penetrates to a depth of about a centimeter into the agar which has been changed to a bluish color.

INOCULATION AND INFECTION

Alternaria brassicae

Laboratory experiments

On Dec. 10, 1915, a water suspension of spores of *Alternaria brassicae*, secured from the University of Wisconsin experimental plot, was made. Two cabbage plants about five inches high in pots were treated as follows: Drops of the spore suspension were placed on the leaves on definite spots with a medicine dropper. With a flamed needle holes were pricked through the epidermis of the leaf through the drop. Four leaves on each plant were thus inoculated, each leaf in one spot. A third plant was similarly treated, distilled water being used in place of a spore suspension, to serve as a control. One of the plants on which the spore suspension was used and the control were placed under separate bell jars, the inside of the jars being sprayed with water. The other inoculated plant was placed nearby, uncovered, in the greenhouse.

After three days the plant placed under the bell jar showed signs of infection at the points inoculated. The areas about the needle puncture were brown. No infection was observed on the uncovered plant, nor on the control. On the next day, four days after inoculation, the disease had made considerable progress in case of the inoculated plant under the bell jar. The leaves were bent downward at the points of infection, and they showed wilt. The areas between the needle punctures in

the same place of inoculation were entirely browned. A fragment from one diseased spot was examined under the microscope, and a large number of spores, many in chains of two, were observed. Eight days after inoculation a successful attempt was made, bythe spore dilution method, to isolate the organism.

Eleven days after inoculation the plant left uncovered, which up to this time showed no sign of infection, was placed under a bell jar after large drops of water had been placed on the inoculated areas. The inside of the bell jar was sprayed with water. Three days later there appeared infection in three areas inoculated. Six days after being placed under the bell jar all four areas inoculated were infected, three areas showing diseased spots about one centimeter in diameter. Nine days after being placed under the bell jar one spot measured nearly two centimeters in diameter. This spot was characteristic of those usually produced by A. brassicae. It showed irregular concentric rings, was brownish at the center with abundant conidia, and had a light yellow margin at the border which shaded gradually into the surrounding tissue. The lower surface was darker in color than the upper. On the whole, the spot was not as dark as is usual on leaves in nature. The other spots were similar in appearance but smaller. The conidia were in chains on short conidiophores which grew in groups from the leaf.

Another experiment was undertaken in which cabbage plants about five inches high in pots were employed, and were

treated as follows: A suspension of A. brassicae conidia in water was made from a strain secured from the University of Wisconsin plot. Abundant spores were assured by the use of the microscope. A very small drop was placed on a definite spot of the leaf and with a flamed needle one whole was pricked through the epidermis through the drop of suspension. Three such inoculations were made on each of four leaves. A second plant was similarly treated, but the drops of suspension were increased by adding distilled water with a dropper. A third plant was treated likewise, but distilled water was used instead of a spore suspension. This plant served as a control. The pots were then placed on window panes and covered with separate bell jars, a small amount of saturated cotton being placed under each. The chamber in which was placed the plant having small drops on the leaves was not kept as moist as the other two.

In twenty-four hours the small drops of water on the leaves on the first plant had dried off. After two days the plant having the large drops of suspension on the leaves showed signs of infection, which appeared not only in the places pricked with the needle, but generally at the margins of the drops of water. Infection was indicated by small brownish spots. The plant having small drops of suspension placed on the leaves did not as yet show infection. Three days after inoculation this plant showed signs of infection in only three places.

After six days from the time of inoculation the plant

receiving the large drops of suspension showed spots from .5 to 1 centimeter in diameter. The plant originally receiving the small drops of suspension showed spots .2 to .3 of a centimeter in diameter in three places. The latter plant was removed from under the bell jar and placed in the greenhouse.

Three weeks after inoculation the plant originally receiving large drops of suspension and remaining continuously under the bell jar with considerable moisture was in a very unhealthy condition. The leaves affected with A. brassicae were entirely destroyed. The plant was not kept any longer. The control plant up to this time showed no signs of infection due to A. brassicae.

Four weeks after the time of inoculation the three small diseased spots on the plant originally treated with small drops of suspension appeared light grayish in color and resembled insect injuries, but a few very small spots in each of the diseased areas remained dark. The affected leaves were now the lower ones of the stem. The plant was very vigorous, thus showing that a diseased plant may recover in a dry atmosphere. This also proved to be true in other similar experiments with A. brassicae.

A large cabbage plant originally intended for the production of seed pods, and which was growing from the ground in the greenhouse was inoculated as follows with a spore suspension of A. brassicae secured from the University of Wisconsin experimental plot. From an atomizer a stream of water was di-

rected against a definite spot on the leaf until the effect of the fatty material on the leaf surface was overcome and a drop adhered to the spot to be inoculated. The tip of a medicine dropper was placed in molten paraffin and then allowed to cool, care being taken that the hole in the dropper was not closed. This was done in order to lessen adhesion so that a small drop of suspension might be placed on the spot selected for inoculation. A small drop of spore suspension was then placed on the moistened spot of the leaf. In subsequent experiments this method was employed unless otherwise described. Three spots on each of two leaves were thus inoculated. The epidermis of the leaves was not injured by needle punctures or otherwise at the points of inoculation. The places inoculated were marked by dots of India ink about them. The plant was then covered by a chamber having three glass sides. The inside of the chamber was well sprayed with an atomizer, and a vessel containing water was placed in the chamber.

Four days after inoculation there was evidence of infection because of small dark spots in the places inoculated. Nine days after inoculation both of the inoculated leaves had dropped off the plant due to soft rot. Fragments were cut from the infected areas. They were treated for one minute in 1-1000 mercuric chloride, rinsed in three changes of sterile water, and placed on sterile potato agar in a petri dish in an attempt to recover the organism. The fungus developed from the fragments, and recovery of the organism was successful.

On Apr. 14, 1916, a kale plant about six inches high and growing in a pot was inoculated by placing drops of spore suspension of A. brassicae on definite spots on several of the leaves, which were uninjured. The spores were of the strain secured from the University of Wisconsin experimental plot. The plant was placed in a moist chamber in the greenhouse. In the moist chamber was placed a saturated handful of cotton, and over the top was placed a sheet of paper to prevent too high a temperature. In case an inoculated area became dry more water was supplied by means of a medicine dropper.

The moist chamber used was of a form especially suited to the purpose, and was made by the writer. The framework was of white pine. The four corners were made of 1-1/2 inch timber, the inner corners being sawed out with a circle saw so that the thickness on each side was 7/8 inch. The four sides consisted of panes of glass 16 inches high and 14 inches wide. The top contained a pane of glass 14 x 14 inches. The accompanying diagram shows the construction in detail. The mortise-and-tenon joints were glued. Strips of cypress were nailed around the bottom of the frame to give lasting quality. This sort of a chamber proved to be well suited for general laboratory as well as for field purposes, and as a consequence several more were made.

Six days after inoculation there was evidence of infection on the kale plant in several of the places inoculated. Sixteen days after inoculation there were a number of diseased

spots on the leaves resembling those due to A. brassicae, two of them being about two centimeters in diameter. The spots contained abundant conidia. By the spore dilution method recovery of the organism was secured.

By methods very similar to that just described the writer has succeeded in producing the disease on a large number and variety of cultivated crucifers by using spores of A. brassicae isolated from cabbage leaves obtained from the University of Wisconsin experimental plot (Fig. 13, 14). Without experiencing any unusual difficulty reisolations were made from several varieties of cabbage - including red cabbage - kale, kohlrabi, and cauliflower. Spores are usually found on both sides of the diseased area, and infection may take place from the lower side as well as from the upper side of the leaf.

The writer was unsuccessful in getting infection of A. brassicae on red clover, cucumber, muskmelon, watermelon, and horse-radish, although these plants were placed under the same conditions as cabbage and cauliflower plants, and at times in the same moist chamber.

In order to determine whether the Alternarias received from the various states were identical to A. brassicae isolated from the University of Wisconsin experimental plot, a series of Hollander cabbage plants was inoculated. The leaves were uninjured. The same method described in the experiment with the kale plant was employed. The plants were vigorous and fairly uniform in size, being about eight inches high in pots.

Four plants were placed under each of two moist chambers, while the plant inoculated with the strain from the University of Wisconsin plot was placed in a separate moist chamber.

Four days after inoculation there were signs of infection on all of the nine plants representing nine strains. Seventeen days after inoculation spots which resembled those due to A. brassicae were not lacking on any of the plants. No notable difference in virulence, in progress of the disease, or in appearance of the spots was manifest. Reisolations were made from all the different strains. Measurements from the spores on the leaves were made, and an average of the largest spores was as follows:

Ames, Iowa	62.8 x 12.8μ
Wooster, Ohio	65.8 x 13.8
Farmingdale, Long Island	70.5 x 14.4
Rochester, New York	73.8 x 14.8
Burlington, Vermont	70.2 x 14.2
Racine, Wisconsin	62.2 x 13.0
Norfolk, Virginia	67.4 x 14.6
Morgantown, West Virginia	60.5 x 13.0
U. of W. experimental plot	68.6 x 14.0

It will be seen that the average diameter of these conidia is not as great as the average diameter secured from spots on leaves in nature. This is due to the fact that the conidia are comparatively young and septation is not complete. On older spots secured by artificial inoculation conidia of

greater diameter may be found. Otherwise the conidia of each strain were very similar to those of the strain secured from the University of Wisconsin plot. To this strain the strain secured from Experiment, Georgia, was previously compared and found to be identical.

Field experiments

During the summer of 1916 persistent efforts were made to secure infections by artificial inoculations on cultivated cruciferous plants growing in the field. The following kinds of plants in considerable numbers were grown on the University of Wisconsin experimental plot: Two varieties of early cabbage, three varieties of late cabbage - including red cabbage -, cauliflower, collards, kale, kohlrabi, broccoli, savoy, and Chinese cabbage.

The summer, which was very hot and up to the last part of July very dry, was, on the whole, unfavorable to the development of A. brassicae as well as to a large number of other fungi. Although the work was begun early in spring and continued until late in fall, and every possible means was applied, no positive evidence of infection due to artificial inoculation can be given. Only during the fall when rainfall was abundant was the writer able to isolate A. brassicae from about a dozen spots, probably due to natural infection, on cultivated crucifers growing in the field.

Infection in storage

On Dec. 9, 1915, the outer leaves of four cabbage heads were carefully removed. The leaves beneath were washed with a 1-1000 mercuric chloride solution, and these leaves were in turn removed so that a clean, healthy leaf surface appeared. The four cabbage heads were then inoculated by placing drops of an aqueous spore suspension on the leaves and pricking through the drops with a flamed needle. A clean piece of towel paper was then placed on each of four clean panes of window glass. Upon the towel paper was placed a clean cabbage leaf, and upon the cabbage leaf was placed the inoculated cabbage head. A small handful of saturated cotton was placed beside the cabbage head which was then covered with a low bell jar. All four cabbage heads were similarly treated, except that the cotton in one case was less moist than in the case of the other three.

The cabbage head in the bell jar provided with a small amount of moisture and one of the others was placed in the greenhouse, the temperature ranging from about 17 to 24°C. One of the other two heads was placed in the cellar where the temperature varied considerably, but no data as to temperature were taken. The remaining cabbage head was placed in the laboratory refrigerator where the temperature varied from about 8 to 12°C.

Five days after inoculation the cabbage placed in the cellar and the one placed in the refrigerator showed slight

browning at the points of puncture made by the needle. The two cabbages placed in the greenhouse showed considerable infection and the leaf tissues between the punctures at the same place of inoculation were brownish due to a diseased condition. In the bell jar in the greenhouse originally receiving the greater amount of humidity,, the humidity was increased at this time, while there was no addition of moisture in the case of the one having a smaller amount of humidity originally.

Three weeks after inoculation the cabbage in the greenhouse in the chamber kept the more humid was in a bad condition. One of the outer leaves was almost entirely destroyed by A. brassicae and by bacteria. The leaf beneath was infected and showed a brownish spot about five centimeters in diameter. By means of potato agar plate cultures A. brassicae was recovered. This cabbage head was not kept any longer.

The cabbage kept the less humid under the bell jar in the greenhouse showed large spots of infection. However, it was not so badly diseased as the former one. It was evident that much moisture favors the progress of the disease.

Five weeks after inoculation the cabbage kept in the refrigerator showed a spot, circular in outline, about six centimeters in diameter and almost covered with brown spores. Around this spot was a margin about one centimeter wide of a lighter color than the center and contained no spores (Fig.). Whether this margin was due to the mycelium of A. brassicae, or whether it was due to bacteria was not determined. The dis-

eased condition of this cabbage closely resembled that observed on cabbages brought from Racine, Wis. to the laboratory by Mr. R. E. Vaughan.

The cabbage kept in the cellar also showed spots of infection several centimeters in diameter. However, the progress of the disease was slower than under the conditions in the refrigerator. The experiment shows that in storage both moisture and temperature have considerable influence upon the disease due to A. brassicae on cabbage.

Macrosporium herculeum

Laboratory experiments

On definite spots on the leaves of three cabbage plants in pots and about 7 inches high, drops of water were caused to adhere by directing a stream of water on the spot from an atomizer. Otherwise the leaves were untreated. The moistened areas on two of the plants were inoculated with a spore suspension of Macrosporium herculeum in water. The strain was secured from the University of Wisconsin experimental garden. The places inoculated were marked by dots of India ink. The third plant was similarly treated, but distilled water was used instead of a spore suspension. The experiment was begun on Feb. 8, 1916. The plants were placed in separate moist chambers in the greenhouse. A handful of wet cotton was placed in each moist chamber, and was kept moist throughout the experiment.

Forty hours after inoculation there were visible signs of infection. This was indicated by small brown spots at the margins of the drops of suspension. Eight days after inoculation there were several spots on each of the plants at the points of inoculation, some being from 5 to 8 millimeters in diameter. A number of spores which appeared to be those of M. herculeum were found on one of the diseased areas. Fifteen days after inoculation some of the diseased areas were two centimeters or more in diameter. The spots were circular, grayish in color, and marked by concentric rings. They were bordered by a yellowish-green margin which shaded gradually into the healthy tissue of the leaf (Fig. 16). On some of the spots conidia were found, but they were comparatively few in number. The conidia were borne on straight or bent conidiophores, 25-32 x 5-6μ, which grew from the leaf in small groups. Most of the spots contained no conidia. Isolation by the spore dilution method on potato agar was successful. The control plant showed no disease due to M. herculeum.

On the same day, Feb. 9, 1916, a very similar experiment was begun, the cabbage plants being of approximately the same size. The strain of M. herculeum, however, was secured from Wooster, Ohio.

Forty hours after inoculation small brownish spots at the margins of the drops of suspension indicated infection. Eight days after inoculation the disease had made much less progress than that due to M. herculeum secured from the University of Wisconsin experimental garden above described, and this

condition was noticeable throughout this experiment as well as in subsequent experiments. It appears that the strain of M. herculeum secured from Ohio was not as virulent as that secured from Wisconsin, but no other difference was noticeable. Twenty days after inoculation only a very few spores appeared on some of the diseased areas, while on others more were found. Isolation by the spore dilution method was attempted, but this was unsuccessful.

Beginning on Mar. 2, 1916, the above experiments were repeated, the same strains of M. herculeum being employed, and the plants being of about the same size.

In general the results were the same as in the former experiments. As before, the strain of M. herculeum from the University of Wisconsin experimental garden was more virulent than that from Ohio. It was noticed in this experiment that the spots on the lowest leaves on the plants increased most rapidly in size, while those spots on the uppermost leaves increased most slowly in size. This condition existed alike on the plants inoculated with the strains from Wisconsin and Ohio. As in the previous experiments, the spores on the diseased areas were comparatively few in number in case of both strains. It probably is on this account that the spots have a grayish appearance, as the light brown spores do not cover much of the surface of the diseased area. Isolation of the organism by the spore dilution method on potato agar from the Ohio strain was successful, as was that from the Wisconsin strain.

At the time of year when the above experiments were run the greenhouse was at a temperature of from 16 to 22°C. during the daytime, and usually considerably lower at night. With mild weather early in March, 1916, came a rise in temperature in the greenhouse especially during the night.

By a similar method of inoculation and similar subsequent treatment of plants as described above, experiments were begun on Mar. 15, 1916, on the following plants in pots, the Wisconsin strain of M. herculeum being employed: Cauliflower, kale, kohlrabi, and watermelon. At no time was there any infection on any of the plants. Similarly, on Mar. 19, 1916, the following different kinds of plants were inoculated with the conidia from the Wisconsin strain: Cauliflower, kale, kohlrabi, and horse-radish. Up to June 7 no infection on any of the plants was noticeable. The temperature ranged from 18 to 23°C. in the greenhouse, it being comparatively high during the night. On Mar. 29, 1916, the experiment was repeated with the Wisconsin strain. The plants used were cabbage, kale, kohlrabi, and horse-radish. The results were all negative as before.

On June 7, 1916, another attempt to secure infection with the Wisconsin strain was made. The leaves of cauliflower, kale, kohlrabi, and horse-radish were inoculated in the usual manner. The plants were placed in a moist chamber out of doors on the north side of the pathologium in the shade. At the beginning the temperature was 12°C., and cool weather with occasional rain prevailed for several days following inoculation.

The plants were sprayed several times during the experiment to insure a high humidity.

On the kohlrabi plant several spots due to M. herculeum developed, and isolation by the spore dilution method on potato agar was successful. Only one large leaf spot apparently due to M. herculeum developed on the kale plant. Conidia in small numbers were present, but isolation by the spore dilution method was not successful. No infection due to this fungus developed on either the cauliflower or on the horse-radish plant. The above series of experiments indicates that a rather low temperature is favorable to leaf spot on cultivated crucifers due to M. herculeum.

On Dec. 18, 1916, the uninjured leaves of a cabbage plant about eight inches high in a pot were inoculated with a water spore suspension of a strain of M. herculeum secured from a rutabaga leaf from Wausau, Wis. The plant was placed in a moist chamber and subsequently treated in the usual manner.

Infection was noticeable in three days after inoculation. The fungus seemed as virulent as the strain secured from the University of Wisconsin experimental garden. A large number of spots typical to those produced by M. herculeum developed. Spores were found as usual on both sides of the leaf, but they were not very abundant. Recovery of the organism by the spore dilution method on potato agar was successful.

Field experiments

During the summer of 1916 persistent efforts were made by the writer to produce leaf spot due to M. herculeum on cultivated crucifers in the field. The work was continued from early in the spring until late in the fall. The kinds of plants used for this purpose were the same as those used for experimenting with A. brassicae. Advantage was taken of all kinds of weather conditions as they appeared. In some cases moist chambers were used in the field.

No infections due to M. herculeum were secured. Not a leaf spot, on any of the plants, which contained spores of M. herculeum was found in the plot as the result of artificial inoculation. No spots whatsoever were found in the plot which contained the spores of the fungus.

Infection in storage

Late in the fall of 1916 an experiment was run to find out whether it was possible for M. herculeum to do damage to cabbage in storage. The leaves of three cabbage heads were carefully removed so that a clean surface was presented. With a flamed needle several punctures were made in the leaf of the cabbage head in a definite spot. Several such spots were thus prepared on each of the cabbage heads. A drop of water spore suspension of M. herculeum was placed on the prepared spots on two cabbage heads. Sterile water was used to put on the prepared spots on the third cabbage head to serve as a control.

The cabbage heads were placed on separate pieces of window glass covered by a clean piece of towel paper, and were then covered with low bell jars. They were then placed in the cellar connected with the greenhouse.

About three weeks after inoculation infection was evident on both the cabbages treated with the inoculum. About six weeks after inoculation each of the two cabbages treated with the spore suspension contained several diseased areas. On one of the cabbages one of the diseased areas was about six centimeters in diameter. It was light brown with an abundance of conidia, and to the unaided eye appeared very like the diseased area due to A. brassicae illustrated in Fig. 15. From conidia on this spot recovery of the organism was readily secured on potato agar plates. On one of the spots on one cabbage a dense, tough, elevated, pure white mycelial mat characteristic of the fungus, as described under the topic "cultural characters", and about two centimeters in diameter was observed. The experiment shows that the fungus may cause decay of cabbage in storage. The control cabbage showed no infection due to M. herculeum.

Alternaria Sp.

Laboratory experiments

A cabbage plant, in a pot, about eight inches high was inoculated by placing drops of water spore suspension of Alternaria sp. on the leaves. The epidermis in areas of the spots inoculated was injured by pricking it with a flamed

needle, while other areas were left uninjured. The plant was placed in a moist chamber in the greenhouse as usual, the temperature ranging from 68 to 72°F. No infection was observed.

Another cabbage plant was similarly inoculated and placed in a moist chamber but was placed in another room of the greenhouse where the temperature ranged from 75 to 80°F. Three days after inoculation infection was evident in the needle punctures. The disease, however, made but little progress, and at the end of ten days nothing beyond an incipient infection occurred. By the fragment method isolation of the organism was attempted and was successful. No infections occurred where the epidermis was uninjured.

A similar experiment was run in which one of each of the following plants was inoculated both in injured and uninjured surfaces of the leaf: Cauliflower, kohlrabi, and horse-radish. The plants were placed in a moist chamber at 75 to 80° F. In three days after inoculation incipient infections occurred on the leaves of the cauliflower and kohlrabi plants in the needle punctures only, but the disease made no further progress though the plants were allowed to remain under the conditions mentioned for two months. No diseased condition whatever due to Alternaria sp. was noticeable on the horse-radish leaves.

On May 18, 1917, another attempt was made to secure by artificial inoculation spots on leaves of cultivated crucifers similar to those on leaves collected in the field. The plants used for this purpose were about eight inches high and

growing in pots. Drops of spore suspension were placed on definite areas and the inoculated areas were marked by India ink. The inoculated areas on some of the leaves of each plant were left uninjured, while the epidermis in other areas was injured by pricking with a flamed needle. The following different kinds of plants were used: Hollander cabbage, cauliflower, kohlrabi, rutabaga, and horse-radish. The plants were placed in a moist chamber in the greenhouse.

Incipient infections appeared in three days, after which the progress of the disease was slow. After ten days incipient infections in the areas pricked with the needle were numerous on the cabbage, cauliflower, kohlrabi, and rutabaga plants, but the horse-radish plant showed no signs of infection.

The writer does not consider this Alternaria as a parasite, but rather as a saprophyte which under certain conditions may take on parasitic habits. It appears that a high temperature favors its development, while only a fair amount of humidity is necessary.

Infection in storage

In the fall of 1916, the outer leaves of three cabbage heads were removed so that a clean surface was presented. With a flamed needle several punctures were made in various small areas of the outer leaves. On these punctured areas drops of spore suspension of Alternaria sp. were placed in case of two of the cabbages, while distilled water was placed on

those of the third cabbage which served as a control. The cabbages were placed on clean paper on window glass, and each cabbage was then covered by a separate, low bell jar. The three bell jars with their contents were then placed in the cellar connected with the greenhouse. No data as to temperature were taken, but,on the whole, it was rather high for a cellar.

The cabbages were examined from time to time, but at no time were there any signs of infection due to the fungus under consideration. Unfortunately, the experiment could not be repeated. No definite reasons why the fungus failed to act as a saprophyte in this case can be given, but the writer is of the opinion that the temperature was too low to permit development.

LONGEVITY

Alternaria brassicae

Overwintering of spores in the ground

In order to determine whether it is possible for the conidia of A. brassicae to survive the winter, the following test was made. The leaves of a young cabbage plant in a pot were inoculated with the spores of the University of Wisconsin strain and placed in a moist chamber in the usual manner. After the leaves had developed good diseased spots with abundant conidia, several of the leaves were removed from the plant and packed one on top of the other. One clean, healthy leaf, secured from a cabbage head, was placed on each side of the diseased leaves. Two thicknesses of clean filter paper were then placed around the cabbage leaves, and the whole was surrounded by two or three thicknesses of cheese cloth and bound together with a cotton string, thus making a package about ten inches square and one and one-half inches in thickness. The package was buried seven or eight inches beneath the surface of the soil in the university garden on Nov. 22, 1916, and allowed to remain there during the winter. The winter was severe, the ground being frozen about two feet in depth.

On May 11, 1917, the package was removed from the ground. The cheese cloth showed considerable decay, but the filter paper was not much affected. The cabbage leaves showed considerable disintegration, but the form of the leaves and

most of the substance still remained. Spores of A. brassicae were found in abundance and in a good state of preservation. The normal brown color was not affected. A water suspension of conidia was made, and hanging drop slides were prepared which were examined after twenty-four hours and again after forty-eight hours. It was found by use of the microscope that approximately twenty per cent of the spores had germinated. This seems to prove that the conidia of A. brassicae are able to survive the winter in the ground.

Plates of potato agar containing the spores of A. brassicae were also made by the dilution method. An immense number of colonies appeared. A. brassicae was isolated from the plates, and inoculations on cabbage leaves were made in the usual manner. Infections were noticeable in three days and large diseased areas developed.

Desiccation in relation of viability of spores

No difficulty was experienced in securing agar slope cultures from cultures of A. brassicae which had been standing in the laboratory for months. This applies to all of the ten strains previously mentioned. While making inoculations, tests for viability of the spores were frequently made on hang drop slides, but at all times was the percentage of spore germination found to be high.

On May 23, 1917, plates of potato agar, containing the spores of A. brassicae secured from the University of Wis-

consin experimental plot, were prepared by the spore dilution method. The spores were taken from two different tubes which were made Dec. 16, 1915. On one set of plates made from the spores from one of the tube cultures a great number of colonies of A. brassicae developed; on the other set of plates from the other tube culture a fair number of colonies developed. It is probable that the original number of spores in the latter set was not so great as that of the former. It is evident that the conidia of A. brassicae can endure desiccation for a long time.

In view of the fact that this fungus frequently lives on cabbage in storage as a saprophyte, that the spores may overwinter in the ground, and that the conidia are able to endure desiccation for at least eighteen months, it is evident that the fungus encounters but little difficulty in establishing itself year after year.

Macrosporium herculeum

Overwintering of spores in the ground

In the fall of 1916, a cabbage plant about nine inches high and growing in a pot was inoculated by placing on the leaves drops of spore suspension of M. herculeum of the strain isolated from material from the University of Wisconsin experimental garden. The plant was then placed in a moist chamber in the greenhouse as usual. Although a considerable number of diseased areas developed, only one lower leaf contained a spot on which conidia were produced in abundance, thus making the dis-

eased area light brown in appearance rather than gray. By the side of this spot and confluent with it appeared conidia which resembled, and later proved to be, those of Alternaria sp. previously described under "cultural characters". The leaf was wrapped up in a bundle and buried in the University of Wisconsin garden on the same day and in precisely the same way as described for the leaves of A. brassicae, and upon removing the package from the ground on May 11, 1917, the state of preservation of the leaf was practically the same. The light brown conidia had retained their color.

Due to the entanglement of the conidia among the overwintered hyphae it was almost impossible to secure what may properly be called a spore suspension. Small pieces of the leaf area containing spores of M. herculeum were placed on a slide and torn into fragments by use of a needle and scalpel. These small fragments were suspended in water and placed in hanging drop slides. The conidia did not germinate readily, but on the third day there was unquestionable evidence that about three per cent of the conidia of M. herculeum had germinated, a few spores showing from two to four germ tubes. Due to the intertwining of the overwintered mycelium with the conidia it was difficult to tell with any degree of accuracy what proportion of conidia had germinated, but the estimation was placed at about eight per cent. On account of the large size of the conidia of M. herculeum it is not likely that the writer has made a mistake in the species of spores which showed germination.

The experiment seems to prove beyond a reasonable doubt that this fungus can overwinter in the conidial stage in the ground. The fact that a few colonies, from which the fungus was isolated, appeared on potato agar plates made ffom a spore suspension, or rather a fragment suspension, of the overwintered leaf lends further evidence that the fungus can overwinter in the ground.

Desiccation in relation of viability to spores

At various times during the two years of research the writer made transfers from tube cultures of M. herculeum which had been standing in the laboratory for months. No difficulty was encountered in securing growth. On May 22, 1917, plates of potato agar were prepared from the conidia of the strain isolated from the University of Wisconsin experimental garden and from the Ohio strain, both of which were taken from tube cultures prepared on May 14, 1916. In both cases there appeared to be a lack of vitality of the spores, as development of the colonies was slow. Nevertheless, several colonies of each strain were secured on the plates.

From what has been said regarding this fungus as a saprophyte on cabbage in storage, the overwintering of the conidia, and the time the conidia retain their viability in the dried condition, it is evident that the fungus has no unusual difficulty in overwintering.

Alternaria sp.

Overwintering of spores in the ground

As previously stated in connection with Macrosporium herculeum, spores which resembled those of Alternaria sp. were found on the leaf at the side of the diseased area due to the former fungus. Upon removing the leaf from the ground in the spring some of the spores resembling those of Alternaria sp. were placed in drops of water on hanging drop slides, and attention was given to their germination. The conidia which developed germ tubes were few in number. However, some of the plates prepared to test out the viability of M. herculeum developed colonies of Alternaria sp. in very large numbers, and, being rapid growers, these colonies had to be removed from the plate so as to allow those of M. herculeum to develop.

Desiccation in relation of viability to spores

No difficulty has been encountered in securing growth on potato agar slopes made from spores from tube cultures of Alternaria sp. which had stood in the laboratory for weeks or even months. Nor did the spores taken from such cultures fail to produce incipient infections in wounds of the leaves of cabbage and some other cultivated crucifers.

Although the experiments relative to the longevity of Alternaria sp. are not sufficient to establish definite conclusions, the fact that the fungus was found in great abundance on

plates prepared from overwintered material, the viability of the spores in old tube cultures, and the fact that the fungus has been isolated in the spring of 1916 from spores found on overwintered leaves lying on top of the ground, seems to establish a fair degree of evidence that the spores have but little difficulty in overwintering.

CONTROL MEASURES

Alternaria brassicae

No definite control measures for A. brassicae have been worked out by the writer. Nevertheless, some suggestions may be offered. Since the leaves of cultivated crucifers are covered by a fatty coat and therefore prevent the adhesion of liquids, it is doubtful whether Bordeaux mixture or lime sulphur can be successfully applied. Moreover, the fungus may cause infection from the lower side of the leaf as well as from the upper side, and since it is very difficult to thoroughly cover both sides of the broad leaves with a spray this method of preventing the disease does not seem practical.

From what has been said regarding the fungus as a saprophyte on cabbage in storage, the overwintering of the spores in the ground, and the longevity of the spores in the dried condition, it would seem that in localities where the disease occurs frequently on plants in the field the destruction

of all plants on which the fungus is apt to occur and the destruction of the remnants of cultivated crucifers year after year should be helpful in controlling the disease. The molding of cabbage in storage can probably be prevented if the suggestions by Harter (4) are carefully observed: "(1) Disinfect the storage house by spraying the walls, benches, and bins with Bordeaux mixture; (2) exercise care in handling, so as to minimize injury to the heads; (3) maintain a temperature 1 or 2 degrees above freezing; and (4) keep the humidity as low as possible by proper ventilation of the house with outside air."

Macrosporium herculeum

Nothing has been discovered upon which the writer can base any conclusions regarding the control measures which may apply to this fungus, but if the suggestions given by Harter (4) are followed, M. herculeum will probably give no trouble in storage. The writer is of the opinion that M. herculeum will not cause an epidemic in the field unless the disease is spread by some other form of spores besides the conidia, because the conidia are comparatively few in number and they are so entangled in the mycelium that they probably do not escape as readily as the conidia of most Alternarias.

Alternaria sp.

Although the fungus is very common and is found on a large variety of plants, the writer does not consider it to be

dangerous economically. Alternaria sp. appears to be a saprophyte ordinarily, but under certain conditions it may probably become a wound parasite. A limited number of cabbages secured from storage and examined by the author showed no damage due to this fungus. The single experiment attampted by the writer to determine whether or not the fungus was able to cause decay of cabbage in storage gave negative results. Consequently it is likely that no control measures are necessary.

SUMMARY AND CONCLUSIONS

From the evidence secured during two years of research on the fungi above mentioned, the writer feels justified to make the following statements:

1. Although the literature relative to leaf spot on cultivated crucifers due to Alternaria and closely related fungi is not extensive, the writer is inclined to believe that such diseases exist in practically all humid regions of the countries of Europe. In the United States the leaf spot caused by A. brassicae is evidently widespread in the states east of the Mississippi River, but the writer has no evidence that the disease is generally prevalent in the western states.

2. It seems unlikely that a means can be found to distinguish the various leaf spot diseases by the description of the spots as they are observed by the unaided eye. The so-called concentric rings reveal practically nothing. Such concentric rings have been produced on potato leaves by Dr. L. R. Jones by applying strong fungicides, and he is of the opinion that the rings are the result of the unequal shrinkage in the drying-out of the tissue. Neither does the size or the color reveal the identity of the causal organism. After observing thousands of spots on leaves the writer is unable to distinguish between those due to A. brassicae, M. herculeum, or possibly to another unnamed Alternaria sp. The spots due to any one of them may vary from one or two millimeters (Fig. 14a) to

two centimeters or more (Fig. 14b) in diameter, while the color may vary from light brown to black. Apparently a much better way to determine the identity of the causal organism with a fair degree of accuracy is to place it in culture on a standard medium and watch for the most constant characteristics. Such characteristics cannot be absolutely relied upon, but they serve in a general way to give valuable suggestions, and in case of some of the Alternarias comparatively rapid determinations can be made.

3. The conidia of A. brassicae may be formed in a rather singular manner. The first conidium of the chain is frequently formed by the addition of one cell to another by the process of budding until three cells are united to form one conidium. The second cell of a chain is formed at the terminal end of the first by the process of budding. In case of M. herculeum the second conidium of the chain appears to originate from the terminal end of the first conidium by the process of budding. In case of the third Alternaria the first conidium appears to arise from the conidiophore by the process of budding. The second spore seems to be produced from the terminal end of the first spore by the process of budding, and this process is apparently repeated in subsequent spores of the chain.

4. Of the leaf spot diseases which have come under the observation of the writer that due to A. brassicae was by far the most abundant and most widely distributed through the United States east of the Mississippi River. A. brassicae must

be considered as a parasite, but it is not likely to cause an epidemic on cruciferous plants. Apparently this fungus may cause considerable damage to growers of cauliflower and cabbage on the growing plants as well as on cabbage in storage. M. herculeum, too, must be considered as a parasite, but no reports of damage caused by this fungus are available, and apparently there is no danger of it causing an epidemic. Another undetermined Alternaria, here referred to as Alternaria sp., was found associated with a definite leaf spot very similar to that caused by A. brassicae. This fungus was found on the leaves of cabbage and various other cultivated crucifers, but its pathogenicity is doubtful. Hot weather with a fair degree of humidity seems to favor its development.

5. Alternaria brassicae caused considerable damage to cabbage in storage. The outer leaves are covered by a dark mold which greatly reduces the economic value of the cabbage heads affected. Macrosporium herculeum possesses the power to cause damage in storage, but thus far no serious damage due to the fungus has been reported. The fungus here referred to as Alternaria sp. does not appear to possess the power to cause damage in storage.

6. Sanitary methods of farming are the only suggestive control measures in case of damage to growing plants in local areas. The methods for control of damage to cabbage in storage outlined by Harter (3) are probably practical.

7. The way in which the fungi above mentioned enter the host tissue has not been determined. Apparently the hyphae do not penetrate the living cell walls. It seems likely that some toxic substance kills the cells and makes conditions favorable to the penetration of the hyphae into the leaf tissue.

8. It was found that the conidia of A. brassicae, of M. herculeum and of the fungus here referred to as Alternaria sp. can, when protected by surrounding cabbage leaves, overwinter in the ground. In the dried condition the conidia of A. brassicae were found viable after the cultures had been standing in the laboratory for eighteen months, those of M. herculeum for twelve months, and those of Alternaria sp. for several months. This shows that these fungi have no great difficulty in overwintering.

9. Macrosporium herculeum was originally found on horse-radish, and Alternaria brassicae was several times reported as occurring on this plant. The writer, during the summer of 1916, found the fungus here referred to as Alternaria sp. in great abundance on horse-radish. However, by employing the spores of these fungi the writer was unable to secure even incipient infections on this plant in the laboratory by artificial inoculations, although his efforts were persistent.

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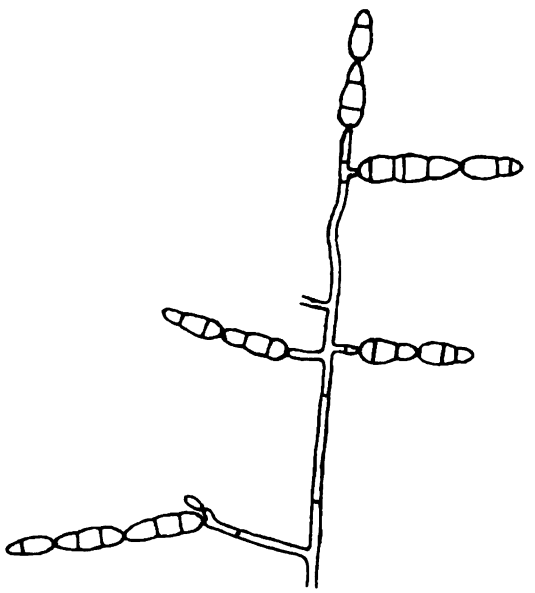
Figures 1 to 3 and 5 to 9 are drawn approximately 430 diameters.

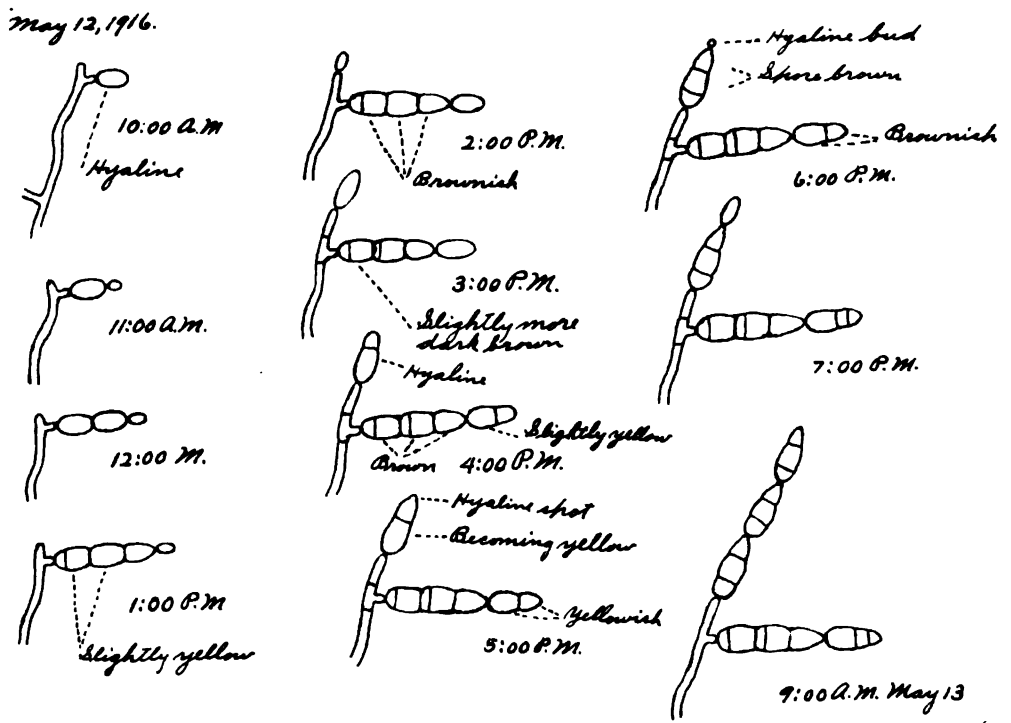
Figure 1



10:00 P.M. May 12, 1916.

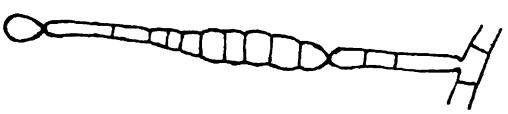
This figure shows various lengths of conidiophores, and in one case two conidiophores nearly opposite each other, on the mycelium of A. brassicae. In the lower part of the drawing it also shows a bud, on a very short conidiophore, which is the beginning of a chain by the side of a chain of three spores. This drawing was made at 10:00 o'clock, and is one of the series made on May 12, 1916, shown in figure 2.

Figure 2



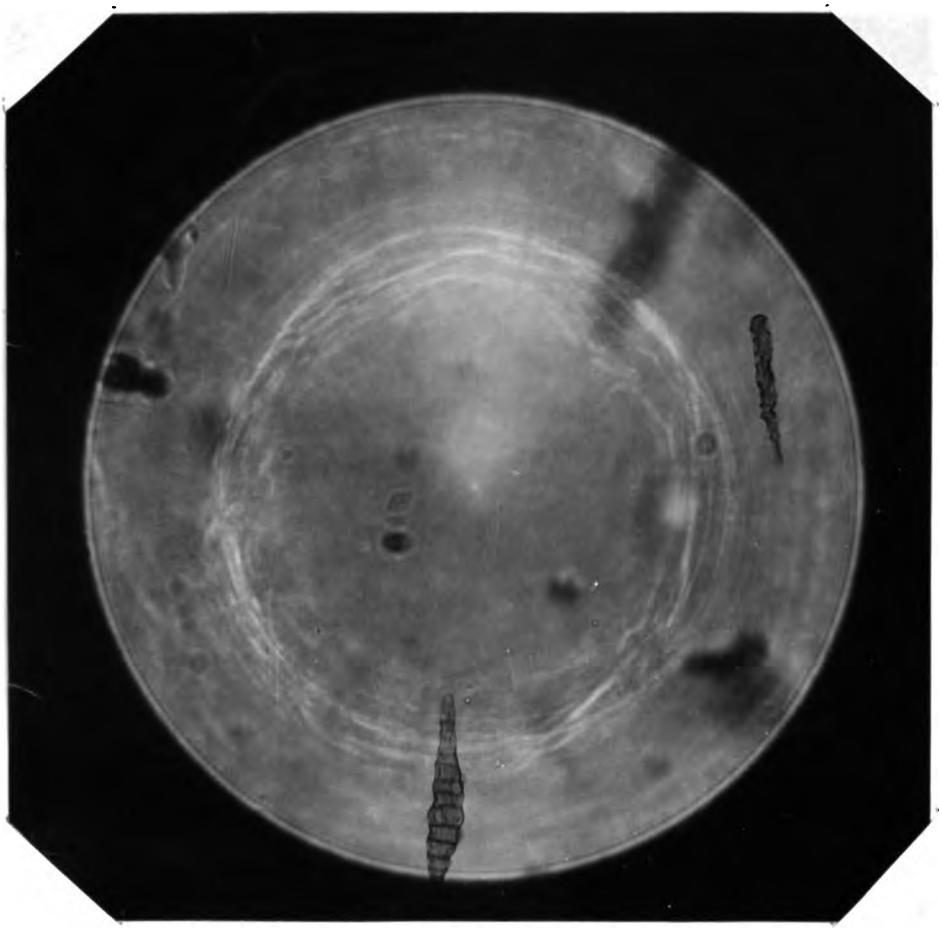
This figure shows conidia formation of A. brassicae, the various drawings, excepting the last, being made at intervals of approximately one hour. A 10:00 o'clock drawing of the same series is shown in figure 1.

Figure 3



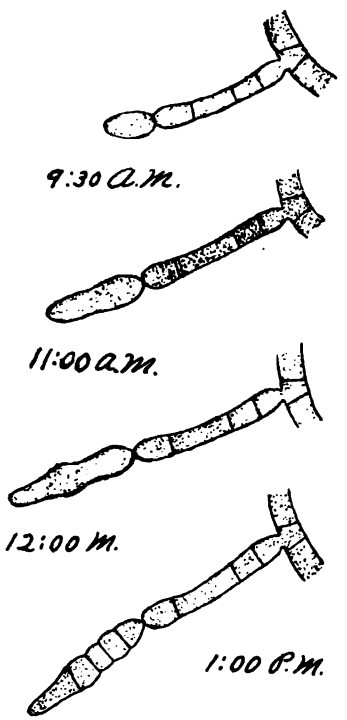
The figure shows a septum in the mycelium on either side of the conidiophore of M. herculeum. It also shows at the end of the beak of the conidium a bud, which is to develop into a second spore.

Figure 4



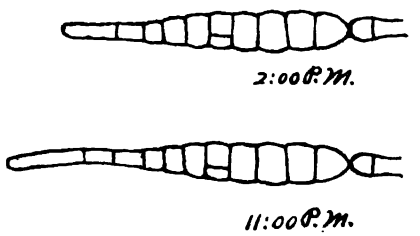
This is a photomicrograph of a conidium of M. herculeum. The conidium was somewhat plasmolized.

Figure 5



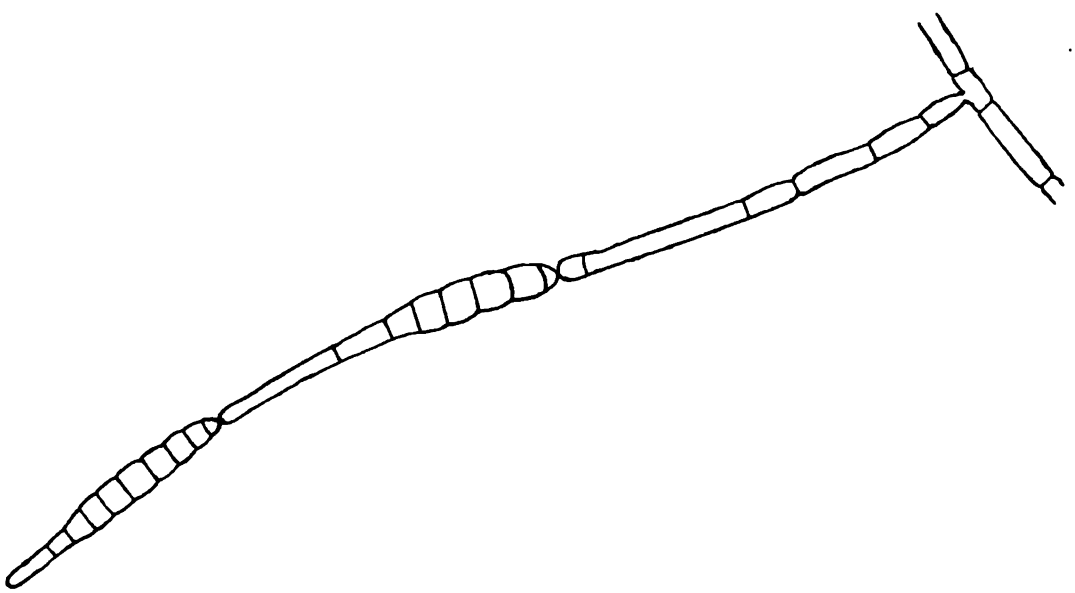
This figure shows spore formation of M. herculeum. The upper stage, 9:30 A. M., shows the conidiophore with a conidium recently budded from the apex, having hyaline walls and granular content. The successive developmental stages follow until 1:00 P. M. when the conidium was multicellular with walls becoming light brown.

Figure 6



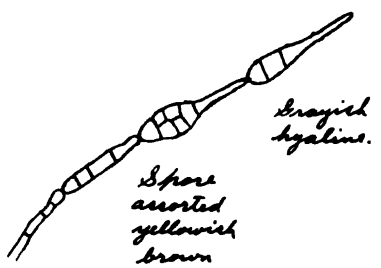
The figure shows a longitudinal septum in a conidium of M. herculeum. It also shows lengthening of the beak and septation in the same.

Figure 7



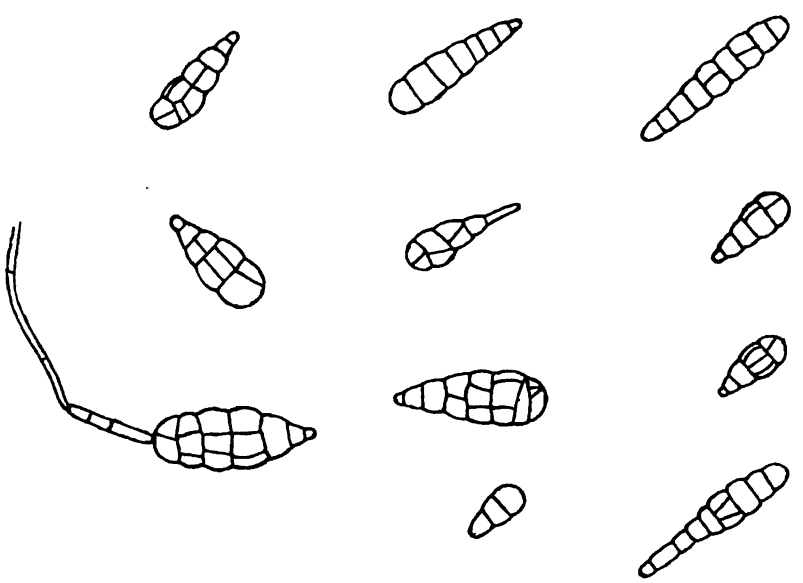
The figure shows a chain of two spores of M. herculeum as observed on the culture slide.

Figure 8



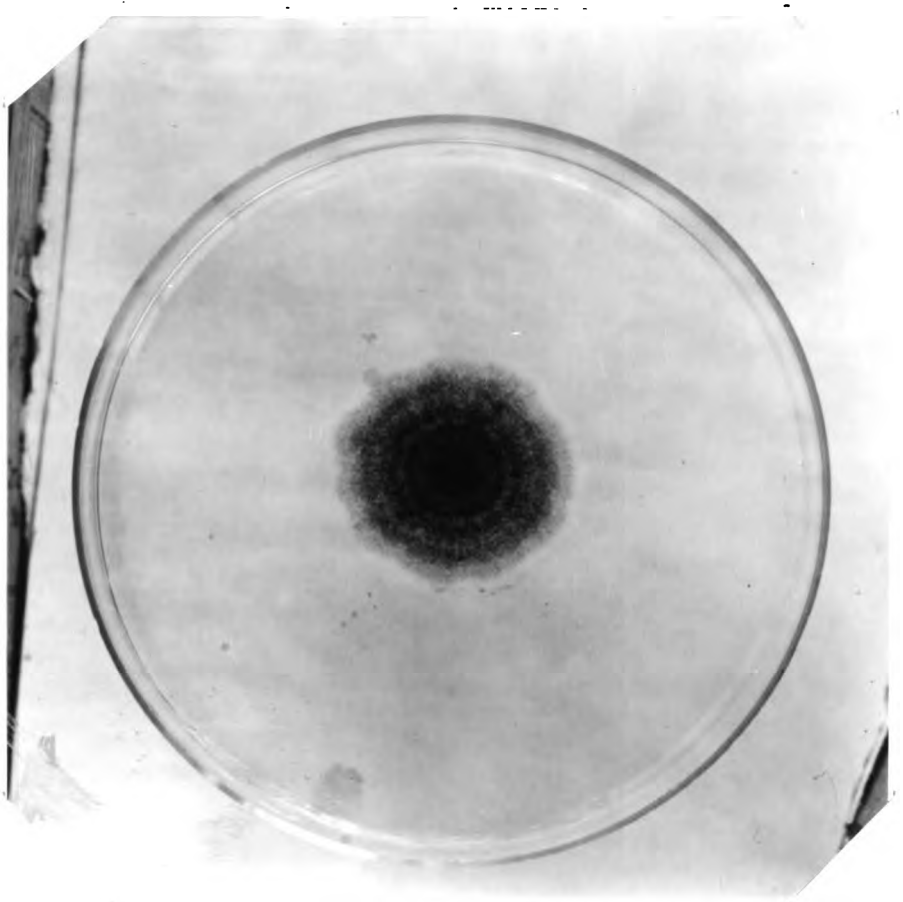
This figure shows a portion of the mycelium, a conidiophore, and a chain of two young spores of Alternaria sp. At this stage the first spore is yellowish brown, while the second is grayish or nearly hyaline.

Figure 9



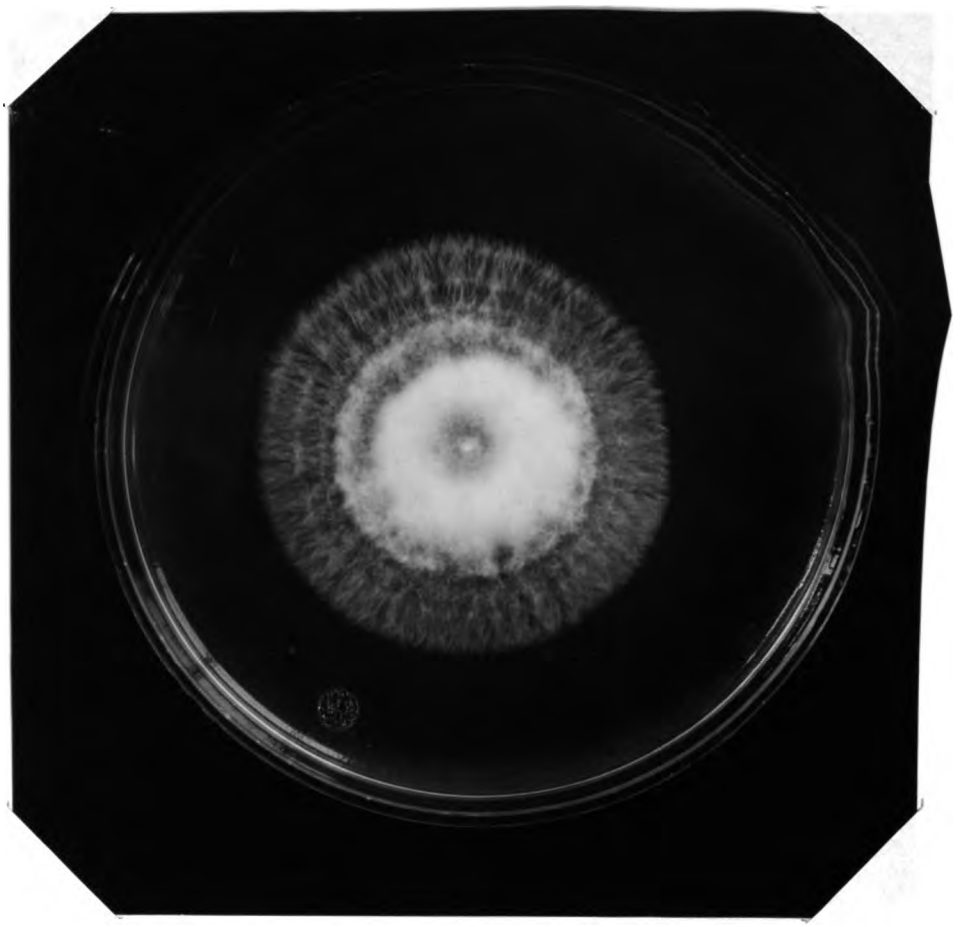
The figure shows various forms of conidia of Alternaria sp. as observed in nature, some being ovoidal in form.

Figure 10



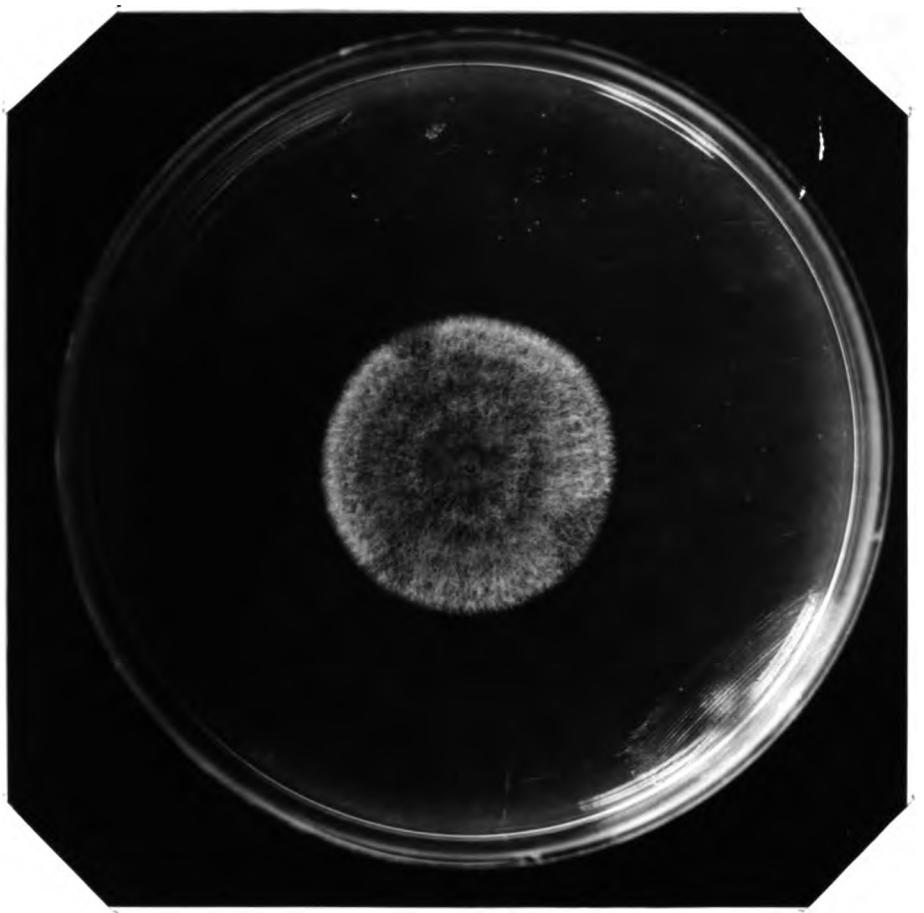
The figure shows a colony of A. brassicae six days after inoculation, on potato agar, bearing abundant conidia.

Figure 11



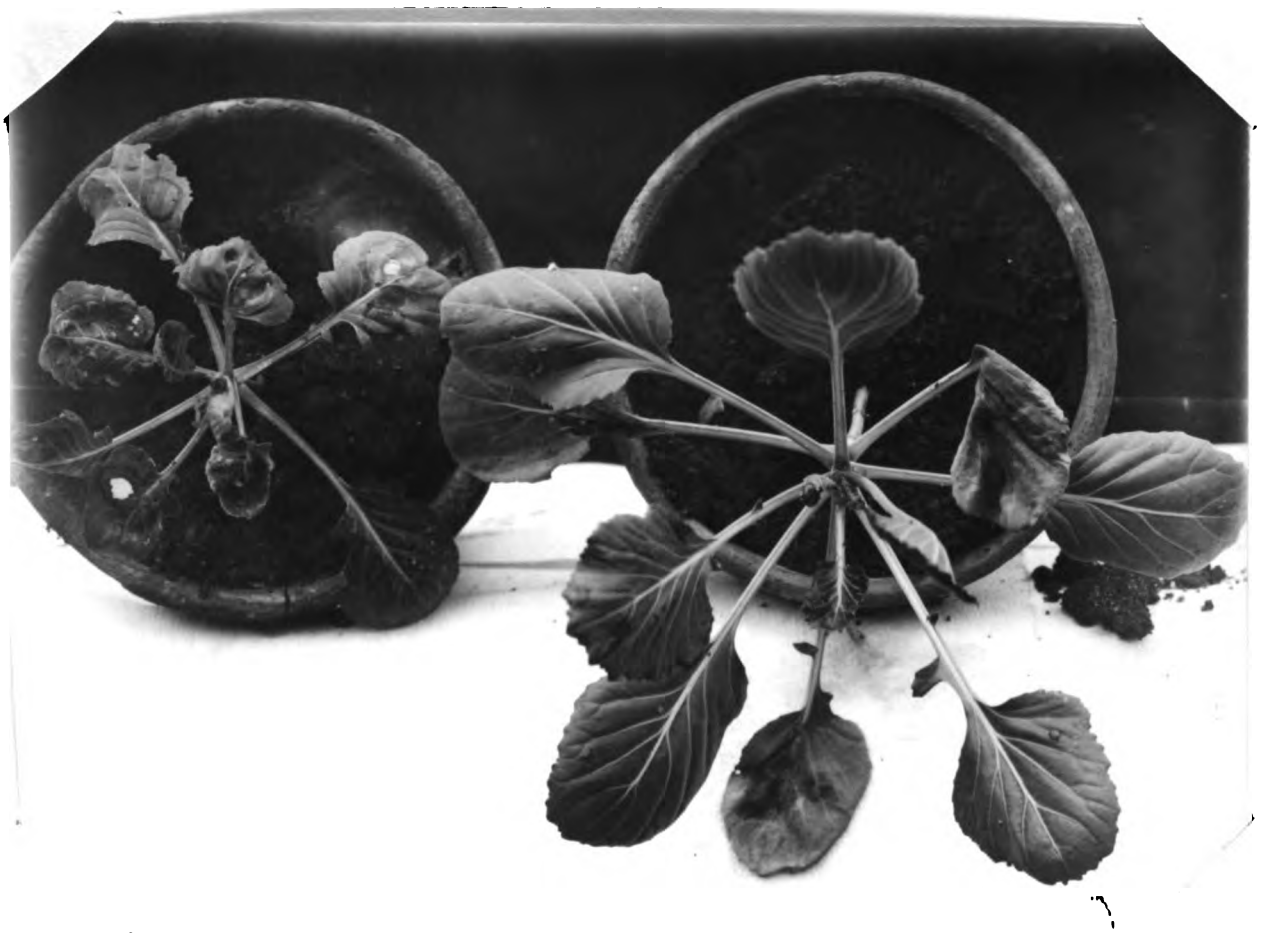
The figure shows a colony of M. hercu-leum eleven days after inoculation, as yet without spores. The photograph, being taken on a black background, represents the margin of the colony as somewhat dark, whereas it is uniformly pure white.

Figure 12



The figure shows a colony of Alternaria sp. eight days after inoculation. Spores are being formed beneath the fluffy, gray mycelium.

Figure 13



This figure shows two pots of diseased cabbage plants, A. brassicae being the causal organism. The left-hand picture shows the centers of two infected areas dropped out. This effect is perhaps due to the work of aphids which were purposely allowed to become numerous. The right-hand plant shows a badly diseased condition in the leaf which is hanging directly downward, and in the leaf extending upward and toward the right. The leaf extending directly toward the left from the stem is affected at the junction of the leaf and petiole, a favorite place of attack in case of young plants.

Figure 14 a

This figure shows a cabbage leaf with one "typical spot" due to A. brassicae on the left. On the right-hand side of the midrib is an infected area, due to the same fungus, which shows a number of black spots less than pin-head size. (Enlarged about one-third).

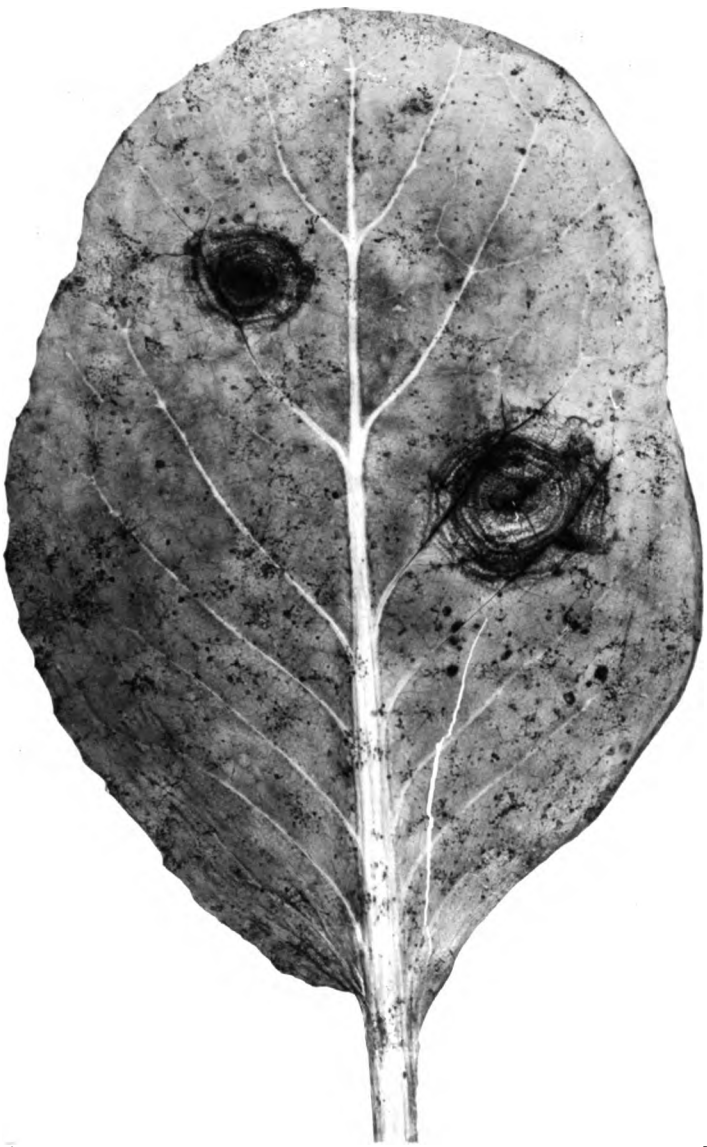
Figure 14 b

The figure shows a cauliflower leaf containing large diseased areas due to A. brassicae.

Figure 15

This figure shows a large brown spot on a cabbage head due to A. brassicae. The cabbage was kept in a refrigerator, and the photograph was taken five weeks after inoculation.

Figure 16



This figure shows two spots produced by M. herculeum resulting from artificial inoculation. The photograph was taken from a dried specimen, and the small dark spots are not indications of infection. (Enlarged about one-half).

Approved L. R. Jones

Date December 3, 1917